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Role of Siderophores in Biocontrol of *Fusarium solani* and Enhanced Growth Response of Bean by *Pseudomonas fluorescens* GL20

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Plant growth-promoting *Psudomonas fluorescens* GL20 was isolated from a ginseng rhizosphere on chrome azurol S agar. *P. fluorescens* GL20 produced a large amount of hydoxamate siderophore in an iron-deficient medium. The siderophore showed significantly high specific activity of 20.2 unit. Using an *in vitro* antifungal test, *P. fluorescens* GL20 considerably suppressed growth of phytopathogenic fungus *Fusarium solani*, inhibiting spore germination and germ tube elongation. In pot trials of kidney beans with *P. fluorescens* GL20, disease incidence was remarkably reduced up to 68% compared with that of *F. solani* alone, and plant growth was also increased nearly 1.6 fold as compared to that of the untreated control, promoting elongation and development of the roots. These results indicate that the plant growth-promoting activity of *P. fluorescens* GL20 can play an important role in biological control of soil-borne plant disease in a rhizosphere, enhancing the growth of plants.

Iron is an essential element for microbial growth, but it is often not readily available to microorganisms in a natural environment. In aerobic conditions, iron is oxidized to extremely insoluble ferric oxyhydroxide polymers at physiological pH (13). The solubility constant of ferric oxyhydroxide is $10^{-38.7}$ (2) and the maximum concentration of free iron is 10^{-18} M at pH 7 (21), a value too low for microbial requirements. To counter these iron-deficient environment, most microorganisms have developed high-affinity iron transport systems, in which siderophores of low molecular-weight high-affinity iron (III)-chelating agents are synthesized, excreted, and consequently the iron complex is transported into the cytosol via a cognate-specific receptor in the outer membrane (14). Although siderophores exhibit a great diversity of structures, most siderophores are classified as either catechol-phenolates or hydroxamates (7).

In recent years, considerable attention has been given to plant growth-promoting rhizobacteria (PGPR). This group of bacteria consists primarily of fluorescent pseudomonads, which are aggressive root colonizers. An important ecological factor in biological control of soil-borne plant disease by PGPR is the production of extracellular siderophores. Siderophores play an essential role in the microbial interactions in a rhizosphere, enhancing the growth of plants and yield of agricultural crops. The mechanism of growth promotion and disease reduction by PGPR is generally believed to be one of antagonistic interactions by siderophore-mediated competition for iron which results in the exclusion of pathogenic and deleterious microorganisms from a rhizosphere. The siderophores of PGPR have been extensively studied, and the introduction of PGPR into a plant rhizosphere to control plant disease or enhance plant growth has been explored (5, 6, 8, 10-12, 15-19, 23-25).

In this study, we describe (i) the isolation of naturally occurring plant growth-promoting rhizobacteria producing siderophores with high activity as biocontrol agents, (ii) the characterization of siderophore activity from selected PGPR, (iii) the mechanism of antagonism of PGPR against *Fusarium solani*, and (iv) the role of siderophores in biological control to fusarial wilt of kidney bean.

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MATERIALS AND METHODS

Chemicals and Glassware

Chrome azurol S (CAS) and hexadecyltrimethylammonium bromide (HDTMA) were purchased from Fluka Chemical Corporation. Anhydrous piperazine, PIPES, 2,3-dihydroxybenzoic acid (2,3-DHBA), deferoxamine mesylate, 8-hydroxyquinoline, 5-sulfosalicylic acid, hydroxylamine hy-

drochlorde, and chitin were purchased from Sigma Chemical Company. All other chemicals were special grade products.

All glassware was rendered iron free with 6 N HCl, rinsed once in distilled water, and finally rinsed in deionized, double-distilled water before sterilization. All media and solutions were prepared with deionized, double-distilled water.

Strains, Media, and Growth Conditions

Siderophore-producing bacteria originally isolated from a rhizosphere in ginseng root-rot suppressive soil, were used in this study. In all experiments, bacteria were grown at 28°C and maintained on a King's B (KB) agar (9). To remove traces of iron, Difco proteose peptone No. 3 was deferrated by extraction with 3% (w/w) 8-hydroxyquinoline in chloroform solution for 2 days as described by Waring and Werkman (22). For the production of chitinase, bacteria were grown on a chitin-minimal agar (CMA) containing 0.5% colloidal chitin, 0.07% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.03% KH_2PO_4, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0001% $ZnSO_4$, 0.0001% $MnCl_2$, and 0.2% $(NH_4)_2SO_4$. *Fusarium solani*, which causes plant root-rot, was kindly provided by the Korea Ginseng and Tobacco Research Institute and was grown at 28°C on a potato-dextrose agar (PDA). All strains were maintained in 30% glycerol at -70°C for long-term storage.

Isolation of Siderophore-producing Bacteria

Bacteria were isolated from roots as well as soils of a ginseng rhizosphere. For the isolation of bacteria from the external root zones (rhizosphere including the rhizoplane), plant roots were gently washed twice in sterile 0.01 M phosphate buffer (pH 7.0-7.2) for 5 min to remove adhering soil, and then shaken for 15-40 min in sterile 0.1% water agar supplemented with 0.1% $MgSO_4 \cdot 7H_2O$ to collect the remaining root soils. For the isolation of bacteria from the internal root zones (endorhizosphere), the adhering soil was carefully removed from plant roots, and the surface disinfected by gently shaking them in 1% sodium hypochlorite (NaOCl) for 30 sec. Roots were then macerated in sterile 0.01 M phosphate buffer (pH 7.0-7.2) with a mortar and pestle. Appropriate serial dilutions of the suspensions from roots as well as rhizosphere soils were plated on chrome azurol S (CAS) blue agar plates as described by Schwyn and Neilands (20), except that KB medium was substituted for the MM9 salts/casamino acids medium. The plates were incubated at 28°C for isolation of siderophore-producing bacteria. An orange halo around a colony on the CAS blue agar plates indicated the production of a siderophore.

Identification of Bacteria

Bacterial isolates were identified by microscopic examination, colonial and cellular morphology, pigmentation, Gram stain, oxidase test, glucose fermentation, and growth on single carbon sources. Gram-negative oxidative bacteria were further identified with API 20NE diagnostic strips (Analytical Products, France) and Gram-negative fermentative bacteria were identified with API 20E diagnostic strips (Analytical Products, France). Gram-positive *Bacillus* species were identified with API 50CH diagnostic strips (Analytical Products, France).

Siderophore Assay

The production of extracellular compounds with siderophore activity was determined using the CAS colorimetric assay (20). This assay utilizes a dye complex of CAS and hexadecyltrimethylammonium bromide (HDTMA) that has a high affinity for iron. The iron dye complex is blue with an absorption maximum at 630 nm using a spectrophotometer (Hitachi U-2000). When a strong chelator, a siderophore, removes the iron from the dye, its colour turns from blue to red by the decolorization of a blue-coloured ferric-CAS complex. In this assay, the siderophore production was estimated by measuring the decrease in absorbance at 630 nm after allowing the solution to equilibrate for 3 h, with deferoxamine mesylate as the standard. The assay mixture consisted of 0.5 ml of CAS assay solution and 0.5 ml of culture supernatant.

A faster-reacting assay solution, termed a CAS-shuttle solution (20), was prepared by adding 4 mM 5-sulfosalicylic acid to the CAS assay solution to destabilize the ferric-CAS complex. The CAS-shuttle assay was conducted at 37°C with a culture supernatant, and the decrease in absorbance at 630 nm was recorded over 20 min. The end-point of the reaction was the time at which no further decolorization of the CAS complex was observed. Total siderophore activity (TSA) was calculated from the initial rate of decolorization, where one unit of activity was a decrease of 0.001 in A_{630} per sec. The total siderophore specific activity was calculated as the TSA units per mg of cell protein present in 1 ml of a culture.

The CAS assay is a universal chemical assay for the detection of siderophores and operates independently of the siderophore structure. The presence of catechol-phenolic type siderophores in culture supernatant was detected by the Arnow phenolic acid assay (1), with 2,3-dihydroxybenzoic acid (2,3-DHBA) as the standard. One unit of activity was μg of 2,3-dihydroxybenzoic acid equivalents per ml. The presence of hydroxamate-type siderophores in culture supernatant was determined by the modified Csaky hydroxylamine/hydroxamic acid assay (4). The assay standard was prepared with hydroxylamine hydrochloride. One unit of activity was μg of hydroxylamine hydrochloride equivalents per ml. The assay was modified as follows. The acid hydrolysis step was conducted by autoclaving at 120°C for 4 h. A 2,5% sodium thiosulfate solution rather than 2% sodium arsenite solution was used as an iodine decolorizing agent. A 0.05% N-(1-naphthyl) ethylenediamine solution instead of a

0.3% α-naphthylamine solution was used as a coupling agent.

Analysis of Antifungal Activity

All bacterial isolates were initially screened for the ability to inhibit fungal growth by extracellular siderophores on agar plates. All isolates were spotted on the center of iron-deficient KB agar plates and of KB agar plates supplemented with 100 μM $FeCl_3$, respectively. After 24 h of incubation, these agar plates were lightly oversprayed with a suspension (10^8 cfu/ml) of *F. solani*. The plates were incubated at 28°C for 4 days. Alternatively, all isolates were inoculated on 2 cm from the edge of KB agar plates prepared as described above. After 24 h of incubation, an agar disk (5 mm in diameter) of *F. solani* inoculum from the leading edge of a mycelium of *F. solani* grown at 28°C for 3 days on PDA was placed on the center of the plates. The plates were incubated at 28°C for 4 days. Inhibition zone of *F. solani* was measured by the distance between the edge of the bacterial colony and fungal mycelium.

Antifungal activities of extracellular siderophores in iron-deficient liquid cultures were investigated. Bacterial isolates were grown at 28°C for 40 h in an iron-deficient KB broth and in a KB broth amended with 100 μM $FeCl_3$, respectively. The bacterial cultures were inoculated with a suspension (10^8 cfu/ml) of *F. solani*. After 4 days of incubation, the fungal mycelia were collected on an oven-dried preweighed paper (Whatman No. 2 filter paper) and dried at 105°C, and dry weights were determined. The inhibition ratios were expressed relative to a control with water.

Bacterial isolates were screened for other antagonistic properties such as antibiotic or chitinase production. Antibiotic production was assayed on PDA plates. The plates were lightly oversprayed with a suspension (10^8 cfu/ml) of *F. solani* and incubated at 28°C for 4 days. The production of an antifungal zone around the bacterial colony suggested the presence of an antibiotic. All isolates were characterized for the lytic ability by the use of a colloidal chitin agar for chitinolytic bacteria. Chitinase production was tested on a chitin-minimal agar (CMA). The production of a clear zone around the bacterial colony indicated the production of a chitinase.

***In vivo* Biocontrol Assay**

To determine the efficacy of siderophore-producing bacteria as biocontrol agents against root-rot disease caused by *F. solani*, kidney bean (*Phaseolus vulgaris* L.) seedlings were used as a test plant. Seeds of kidney bean were surface-sterilized twice with a 10 ml immersion in 1% NaOCl and washed in sterile distilled water. The seeds were germinated for 3 days between sterile moist cheesecloth in the dark at 28°C. A bacterial suspension was prepared by washing cells grown in KB medium at 28°C for 2 days in sterile 0.1 M $MgSO_4$ solution. A spore suspension of *F. solani* was prepared by growing in potato dextrose broth (PDB) at 28°C for 7 days. The culture was sieved aseptically through 10 layers of sterile cheesecloth, centrifuged, and washed five times in sterile 0.1 M $MgSO_4$ solution. The spore suspension was air-dried and stored at 4°C.

Plastic pots (50 × 50 × 50 mm) were filled up to two-thirds with a vermiculite soil, and 3-day-old seedling was transplanted in each pot. A seed cover layer (one-third of the pot's depth) was infested with a preparation of *F. solani* (3×10^9 cfu per gram of soil) and mixed with 5 ml of the bacterial suspension (approximately 1×10^9 cfu/ml) except for a untreated control. The plants were grown in a growth chamber at 22°C with 65% relative humidity (RH) by using a 6 h light-8 h dark cycle. The plants watered daily with 500 ml of plant nutrient solution (3). The plant nutrient solution was prepared by adding 2 ml of the following stock solutions to 1 liter of water: 0.5 M KNO_3, 0.5 M $Ca(NO_3)_2$, 0.2 M $MgSO_4$, and 0.1 M K_2HPO_4. The plants were harvested 14 days after transplanting. Seedlings of kidney bean were examined for the presence of root-rot. The number of healthy kidney beans was recorded for disease incidence. The weights of the whole plant were recorded to measure growth promotion, and the weights compared with that of the untreated control.

RESULTS AND DISCUSSION

Isolation of Siderophore-producing Bacteria

For isolation of plant growth-promoting rhizobacteria (PGPR) as biological agents which suppress soil-borne disease and promote the health and productivity of crop plants, appropriate serial dilutions of washed soils and roots from a ginseng rhizosphere were plated on a chrome azurol S (CAS) blue agar and tested for the presence of a siderophore by observing a color change from blue to orange after 2 or 3 days of incubation. The results of this analysis are shown in Table 1 and Fig. 1. On this medium most isolates, except for those grown without halo production, gave orange-colored haloes around colonies indicating siderophore-mediated iron removal from the ternary complex CAS-Fe(III)-hexadecyltrimethylammonium bromide (HDTMA). Of 24 bacterial isolates, 10 strains produced haloes of larger than 1.0 cm in distance between the edge of the bacterial colony and halo, presumably due to synthesis and excretion of large amounts of siderophores.

Identification of Isolates

Of 5 isolates, which showed strong antifungal activities of extracellular siderophores, isolates GL7, GL17, GL19, and GL20 were identified as *Pseudomonas fluorescens* and isolate GL14 was identified as *Bacillus subtilis* on the basis of their reactions to standard biochem-

Table 1. Siderophore-producing bacteria isolated from a ginseng rhizosphere by CAS analysis.

Strain	CAS blue agar		KB agar
	Growth[a]	Halo formation[b]	Fluorescent pigment[c]
GL1	++	++	
GL2	++	++	
GL3	++	+++	
GL4	++	++	
GL5	++	+	
GL6	++	+++	+
GL7	++	+++	+
GL8	++	++	+
GL9	++	+++	+
GL10	++	++	
GL11	++	+++	
GL12	++	++	
GL13	++	++	
GL14	++	+++	
GL15	++	+	
GL16	++	+	
GL17	++	+++	+
GL18	++	++	+
GL19	++	+++	+
GL20	++	+++	+
GL21	+	+	
GL22	++	++	
GL23	++	+++	
GL24	+	+	
Control	–	–	

Serial dilutions of the suspensions from roots as well as rhizosphere soils were plated on a chrome azurol S (CAS) blue agar and tested for the presence of a siderophore by observing a color change from blue to orange after 3 days of incubation. [a] –, No growth; +, minimal growth; ++, normal growth. [b] –, Absence of halo formation; +, small halos < 0.5 cm wide surrounding colonies; ++, medium halos > 0.5 cm wide surrounding colonies; +++, large halos > 1.0 cm wide surrounding colonies. [c] Diffusible fluorescent pigment on KBA plates.

ical tests from Bergey' s manual and API diagnostic tests.

Production of Siderophores

The production of extracellular compounds with siderophore activity was determined using a CAS colorimetric assay. All bacterial isolates were grown for 40 h at 28°C in an iron-deficient KB medium. The siderophore production in cuture supernatants was estimated by measuring the decrease in absorbance at 630 nm after allowing CAS assay solution to equilibrate for 3 h. The values were compared with optical density (O.D.) of non-inoculated KB medium as the reference. A standard curve for the CAS assay was prepared by analyzing the absorbance at 630 nm with a standard solution of deferoxamine mesylate divided by the absorbance of reference solution (A/A_{ref}) as a function of siderophore concentration. The relationship was proportional to the concentration of siderophore (Data not shown). The CAS

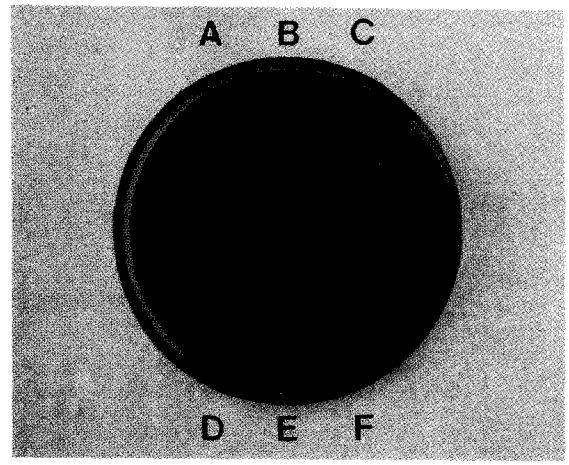

Fig. 1. Growth and siderophore production on CAS blue agar. The plate was spotted with 2 μl of inoculum pregrown in an iron-deficient KB medium and incubated at 28°C for 2 days: A, non-siderophore-producing bacteria; B, GL7; C, GL14; D, GL17; E, GL19; F, GL20.

assay measured the removal of Fe(III) from the blue CAS-Fe(III)-HDTMA complex in the medium, a value of A/A_{ref} smaller than one at 630 nm indicates a positive siderophore reaction. Of supernatants from 24 isolates, 11 showed strong positive reactions, with A/A_{ref} ranging from 0 to 0.25, and 7 showed weak positive, with A/A_{ref} ranging from 0.5 to 1 (Table 2).

The CAS assay is a universal chemical assay for the detection of siderophores. The assay can be applied for any kinds of siderophores. Culture supernatants of all isolates were determined quantitatively by the Arnow test for the presence of phenolate-catechol and by the Csaky test for hydroxamate siderophores. The results of these analyses are shown in Table 2. Of supernatants from 24 isolates, 15 were positive in the Csaky test, indicating the production of hydroxamate siderophores. Additional phenolate-catechol siderophores were produced by 12 isolates. Among them, GL4, GL11, and GL22, produced hydroxamate with phenolate-catechol siderophores. Of 5 isolates with strong positive reactions in the CAS assay, GL7, GL17, GL19, and GL20 produced large amounts of hydoxamate siderophores and GL14 also showed a strong activity of phenolate-catechol siderophore.

Total siderophore activity in culture supernatants of the 5 isolates was confirmed using a CAS-shuttle assay. The assay is based on the acceleration of ferric exchange to siderophore by the addition of 4 mM 5-sulfosalicylic acid to CAS assay solution. The ability of siderophore in the culture supernatants to decolorize the ferric-CAS complex was observed by measuring the A_{630} at 10 sec internals until no further decolorization occurred. As the CAS-shuttle assay was conducted at 37°C with culture supernatant, the reaction was faster than 30°C (Data not shown).

Table 2. Characterization of siderophores in iron-deficient medium.

Strain	CAS liquid assay[a]	Catechol assay[b]	Hydroxamate assay[c]
GL1	0.881^{+}	5.45	0
GL2	0.415^{+}	0	2.74
GL3	0.324^{++}	10.54	0
GL4	0.648^{+}	3.78	2.44
GL5	0.691^{+}	6.72	0
GL6	0.165^{+++}	0	4.03
GL7	0.096^{+++}	0	25.73
GL8	0.144^{+++}	0	3.51
GL9	0.190^{+++}	0	3.65
GL10	0.830^{+}	5.92	0
GL11	0.116^{+++}	11.25	4.16
GL12	0.209^{+++}	0	2.72
GL13	0.840^{+}	3.46	0
GL14	0.112^{+++}	26.36	0
GL15	0.321^{++}	14.35	0
GL16	0.369^{++}	6.48	0
GL17	0.103^{+++}	0	18.75
GL18	0.196^{+++}	0	3.98
GL19	0.096^{+++}	0	7.59
GL20	0.084^{+++}	0	28.54
GL21	0.632^{+}	0	2.51
GL22	0.426^{++}	10.93	1.93
GL23	0.486^{++}	0	2.21
GL24	0.610^{+}	4.02	0
Reference	1.000	0	0

All strains were grown at 28°C for 40 h in an iron-deficient KB medium. [a]Siderophore production was determined by measuring the decrease in absorbance at 630 nm by the method of Schwyn and Neilands. Scaled values of absorbancy/absorbancy reference (A/A_{ref}) at 630 nm: +++, 0.0 to 0.25; ++, 0.25 to 0.5; +, 0.5 to 1.0. [b]The presence of catechol-phenolic type siderophores was detected at 510 nm by the method of Arnow. The value is the concentration of 2,3-dihydroxybenzoic acid equivalents (μg/ml). [c]The presence of hydroxamate type siderophores was detected at 543 nm by the method of Csaky. The value is the concentration of hydroxylamine hydrochloride equivalents (μg/ml). All values are means of three replicates.

Table 3. Total siderophore activity by CAS shuttle assay.

Strain	Total siderophore activity (TSA; U)[a]	Total siderophore specific activity (TSSA; U)[b]	End-point (min)[c]
GL7	15.8	17.6	15~20
GL14	13.8	12.5	15~20
GL17	15.2	15.9	15~20
GL19	14.6	18.2	20~25
GL20	16.5	20.2	15~20

All strains were grown at 28°C for 40 h in an iron-deficient KB medium. Assay was conducted at 37°C with culture supernatant. [a]Total siderophore activity was calculated from the initial rate of decolorization, where one unit of activity was a decrease of 0.001 in A_{630} per sec. [b]Total siderophore specific activity was calculated as the TSA units (mg cell protein)$^{-1}$ present in 1 ml of culture. [c]End-point of the reaction was the time at which no further decolorization of the CAS complex was observed. All values are means of three replicates.

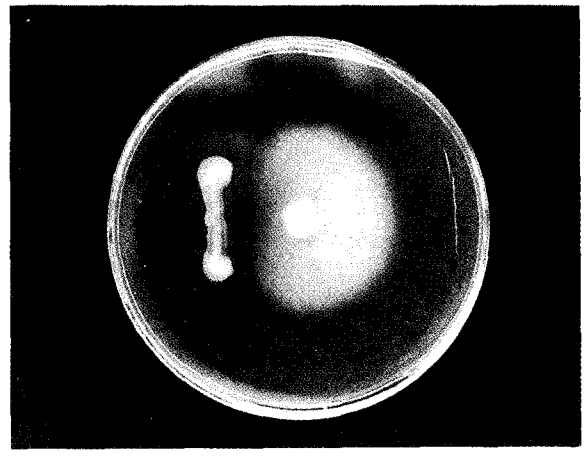

Fig. 2. Petri plate assay for antifungal activity of siderophore-producing *P. fluorescens* GL20 against *F. solani* on an iron-deficient agar.
An iron-deficient KB agar plate was incubated at 28°C for 4 days: Left, *P. fluorescens* GL20; Right, *F. solani*.

Of culture supernatants of the isolates, GL20 showed significantly higher total specific siderophore activity (TSSA) than other isolates with 20.2 unit (Table 3).

Mechanism of Antagonism

The antagonistic properties of 16 isolates against *F. solani* were determined by petri plate assay for antifungal activity (Fig. 2). Among the isolates tested for inhibition zone production on KBA plates with and without 100 μM $FeCl_3$, all isolates showed decreases of inhibition zone in the presence of iron (Table 4). This inhibitory activity generally corresponded with the area of fluorescent pigment diffusion. This suggests that antagonism against *F. solani* is at least partially due to the production of an iron-chelating siderophore.

To determine whether chitinase or antibiotic were responsible for the antagonistic properties in addition to siderophore, we tested for inhibitory activity against *F. solani* in the presence of sufficient iron. PDA was used as a medium to enhance the production of antibiotic because of its high levels of iron which suppress siderophore production. Fluorescent siderophores were not produced by any of the 16 isolates on PDA plates. However, GL14 produced a fungicidal zone against *F. solani* on PDA that apparently resulted from antibiotic production (Table 4). Because the strain inhibited the fungal growth in ferric iron-rich medium, siderophores may not be involved in the antagonistic response. Next, we investigated the possibility of chitinase production from the 16 isolates. Only GL11 showed the production of a clear zone around the bacterial colony on chitin minimal agar (CMA) containing colloidal chitin as a sole carbon source (Table 4).

Antifungal Activity

Siderophore-producing bacteria were screened for their

Table 4. Antifungal properties of siderophore-producing strains against *Fusarium solani*.

Strain	Siderophore (mm)[a]		Antibiotic[b] production *in vitro*	Extacellular[c] chitinase production
	− Fe	+Fe		
GL3	1.0	−	−	−
GL5	1.0	−	−	−
GL7	3.0	0.5	−	−
GL8	1.5	0.5	−	−
GL9	1.5	1.0	−	−
GL11	2.0	1.0	−	+
GL12	1.0	−	−	−
GL13	1.5	−	−	−
GL14	3.0	2.0	+	−
GL15	1.5	−	−	−
GL16	1.5	0.5	−	−
GL17	3.0	0.5	−	−
GL19	2.5	1.0	−	−
GL20	3.5	1.0	−	−
GL22	1.5	−	−	−
GL23	1.0	−	−	−

[a]All strains were spotted on the center of iron-deficient KB agar plates and of KB agar plates supplemented with 100 μM $FeCl_3$, respectively. After 24 h of incubation, these agar plates were oversprayed with a suspension (10^8 cfu/ml) of *F. solani*. Inhibition zone of *F. solani* was measured the distance between the edge of the bacterial colony and fungal mycelium after 4 days of incubation. [b]Antibiotic production was tested on potato dextrose agar (PDA). The production of a antifungal zone around the bacterial colony suggested the presence of an antibiotic. +, antifungal zone present; -, antifungal zone absent. [c]Chitinase production was tested on chitin minimal agar (CMA). The production of a clear zone around the bacterial colony suggested the presence of a chitinase. +, clear zone present; -, clear zone absent. All values are means of three replicates.

ability to inhibit fungal growth. The antifungal activities of extracellular siderophores against *F. solani* on iron-deficient KBA plates were investigated. Of 16 isolates, GL7, GL14, GL17, GL19, and GL20, showed strong antagonistic activities (the degree of antagonism, 2.5 to 3.5 diameter) on the plates (Table 4). The isolates also inhibited fungal growth considerably in an iron-deficient KB liquid medium after 4 days of incubation, compared with that of the untreated control. In the presence of GL 20, *F. solani* did not grow at all in the medium (Fig. 3). Also, spore germination and germ tube elongation were not observed nearly under a phase-contrast microscope (data not shown). These results showed that GL20 produced extracellular siderophore which was strongly inhibitory to growth of *F. solani* in an iron-deficient condition.

Plant Growth-promoting Activity

To determine the efficacy of siderophore-producing strains as biocontrol agents, the cells of the 5 isolates were treated in pots containing kidney beans (*Phaseolus vulgaris* L.). *F. solani* is the causal agent of root rot of kidney bean. The disease is characterized by reddish-

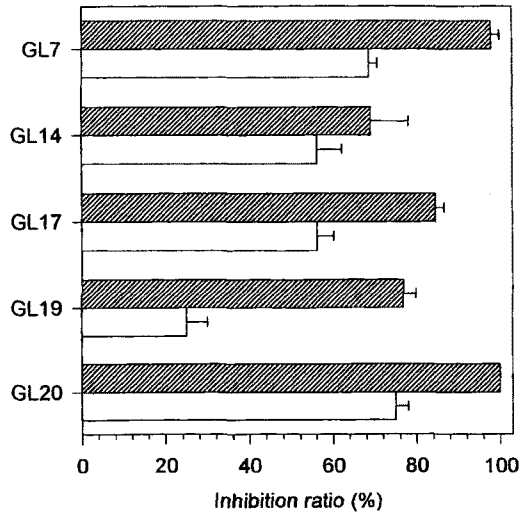

Fig. 3. Antifungal activity of siderophore-producing strains against *F. solani* in iron-deficient liquid cultures.
Bacterial strains were grown at 28°C for 40 h in an iron-deficient KB broth (▨) and in a KB broth (□) amended with 100 μM $FeCl_3$, respectively. The bacterial cultures were inoculated with a suspension (10^8 cfu/ml) of *F. solani* and dry weights of the cultures were determined after 4 days of incubation at 28°C. Each data point represents the mean of three replications; vertical bars represent standard deviations.

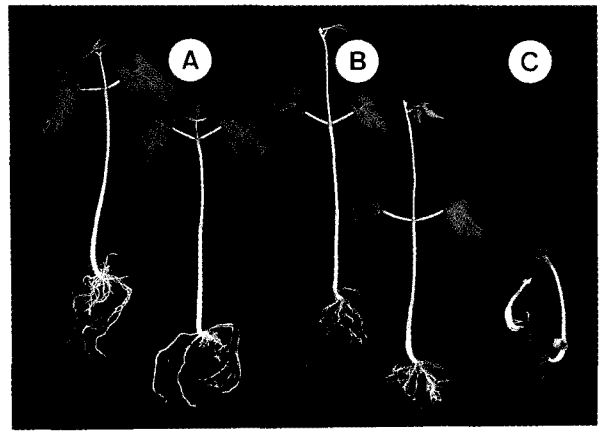

Fig. 4. Growth response on root of kidney bean by *P. fluorescens* GL20.
The plants were harvested 14 days after transplanting: A, *F. solani* with *P. fluorescens* GL20; B, None; C, *F. solani*.

brown lesions that develop on the hypocotyl and lateral roots. The effect of inoculation with 5 isolates on the root rot caused in kidney bean by *F. solani* was examined. After 3 days of seed emergence, each seedling infected with *F. solani* rapidly developed a red-brown lesion at the root-stem interface (Fig. 4C). After 14 days, the disease incidence caused by *F. solani* alone reached to 72% (Fig. 5). Application of the isolates resulted in significant suppression of the disease as compared to that

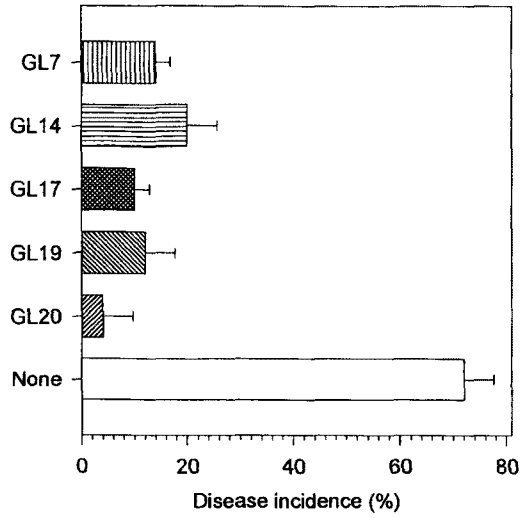

Fig. 5. Disease suppression on fusarial wilt of kidney bean (*Phaseolus vulgaris L.*) by siderophore-producing strains.
Plastic pots (50×50×50 mm) were filled up to two-third with a vermiculite soil, and 3-day-old seedling of kidney bean was transplanted in each pot. A seed cover layer (one-third of the pot's depth) was infested with a preparation of *F. solani* (3×10^9 cfu per gram of soil) and mixed with 5 ml of bacterial suspension (approximately 1×10^9 cfu/ml). Each data point represents the mean of three replications of 50 seedlings each; vertical bars represent standard deviations.

Table 5. Plant growth response of *F. solani* infested-kidney bean by siderophore-producing strains.

Treatement	Average weight per plant (g)	Increase compared to control (%)
Control	6.5	100
GL7	8.2*	126
GL14	7.2	110
GL17	8.8*	135
GL19	7.5	115
GL20	10.3*	158

All seedlings were infested with a preparation of *F. solani* (3×10^9 cfu per gram of soil) and mixed with 5 ml of the bacterial suspension (approximately 1×10^9 cfu/ml) except for the untreated control. The plants were harvested 14 days after transplanting, and the weights of the whole plant were recorded. Each value is the everage of 50 seedlings. *indicates significant increase compared to control (P=0.05).

of *F. solani* alone (Fig. 5). Disease incidence was reduced up to 68% by co-inoculation of seeds with GL20 (Fig. 5). In addition, root development of the kidney beans was enhanced, and plant weight was also considerably increased by comparison with that of the untreated control (Table 5). As a result, GL20 occurred significant increases in plant growth, promoting elongation and development of the roots (Fig. 6). These results indicate that the plant growth-promoting activity of GL20 on kidney bean results from the potential biocontrol

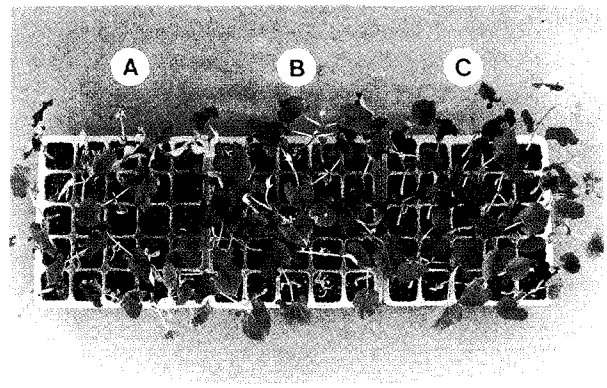

Fig. 6. Enhanced plant growth of kidney bean by *P. fluorescens* GL20.
The plants were harvested 14 days after transplanting: A, *F. solani*; B, None; C, *F. solani* with *P. fluorescens* GL20.

ability of the strain to suppress *F. solani* by production of siderophore.

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