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Antifungal Mechanism of *Pseudomonas stutzeri* YPL-1 for Biocontrol of *Fusarium solani* causing Plant Root Rot

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식물근부균 *Fusarium solani* 에 대한 *Pseudomonas stutzeri* YPL-1의 생물학적 방제기작

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For the selection of powerful antagonistic bacterium for biological control of soilborne *Fusarium solani* causing root rot of many important crops, the best YPL-1 strain was selected among 300 strains of bacteria isolated from rhizosphere in ginseng root rot-suppressive soil. The strain was identified to be a species to *Pseudomonas stutzeri*. With *in vitro* fungal inhibition tests, antagonistic substance of *P. stutzeri* YPL-1 against *F. solani* was presumed to be heat unstable, macromolecular substances such as protein. Also, it was shown that antifungal activity of *P. stutzeri* YPL-1 increased in proportion to its chitinase production. *P. stutzeri* YPL-M122 (*chi*$^-$, *lam*$^-$) which was deprived of the productivity of chitinase and laminarinase by NTG mutagenesis had lost antifungal activity, completely. And *P. stutzeri* YPL-M153 (*chi*$^-$) had only 4.1% of its antifungal activity. *P. stutzeri* YPL-1 was not able to produce any extracellular siderophore in iron-deficent minimal medium. It is confident that the antifungal mechanism of *P. stutzeri* YPL-1 for biocontrol of *F. solani* depends on lysis rather than antibiosis: the mechanism of lysis appears to involve enzymatic degradation of the cell wall components of *F. solani* by hydrolytic enzymes of more chitinase and less laminarinase.

In recent years, the study of microbial antagonism has led us to increse interest in the case of microorganisms for the biological control (1-3) rather than chemical control (4) of soilborne plant pathogens, because it has not linked with environmental contamination by residual problems and phytotoxicity. Antagonism, responsible for some types of biological control of plant root surface, may operate by antibiosis, competition, and exploitation (predation, hyperparasitism ; lysis). Three mechanisms of pathogen suppressions have been proposed. The first proposal is that the bacteria produce

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tion was carried out at 50 °C for 4 hr. The reaction port for the role of antibiotics in biological control has mainly come from studies that have shown correlations between bacterial inhibition of pathogens *in vitro* and disease suppression in the soil. However, the importance of antibiotics in these interactions between bacteria and pathogens in the rhizosphere is much less clearly established, partly because antibiotics have never been detected directly in natural rhizosphere soil. The second mechanism depends on production of siderophores (microbial iron transport agents) by plant growth-promoting rhizobacteria (PGPR) (9-12). Siderophores of PGPR in the rhizosphere under iron deficient environment could efficiently chelate environmental iron and inhibit the growth of native microflora including root

pathogens. The third mechanism depends on lysis of soilborne plant pathogenic fungi appears to involve the enzymatic hydrolysis of glucan and chitin which were composed of the wall of fungal hyphae (13-16). They have been shown to produce hydrolytic enzymes after induction by the appropriate substrates (17-19). Lysis of propagules in soil is a logically satisfying method of biological control since it could reduce inoculum density. They seem to play an important role in microbial equilibrium and can serve as a powerful tool for biocontrol of soilborne pathogenic fungi.

In this study we describe: (i) the isolation, selection, and identification of antagonistic bacteria for biocontrol of soilborne *Fusarium solani* causing plant root rot, (ii) the antifungal mechanism of antagonistic bacterium for biocontrol of *F. solani*.

Materials and Methods

Microbial strains and culture conditions

All antagonistic bacteria were isolated from rhizosphere in ginseng cultivated soil in Yeungpung-gun, Korea. To isolate bacteria, rhizosphere soils were macerated in 0.01 M phosphate buffer (pH 7.2) with mortar and then serial dilutions were plated on nutrient agar (NA). Fungal pathogen used in this study was *Fusarium solani* causing root rot of various economic plants. Culture of *F. solani* was maintained on potato dextrose agar (PDA). For the production of antagonistic substances, isolates were cultured at 30°C on a rotary shaker in chitin-peptone medium (pH 6.8) containing 0.5% glucose, 0.2% peptone, 0.2% chitin, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.05% NaCl. The culture supernatants were used for assay of antifungal activities to *F. solani*.

Identification of selected bacterium

Identification of the selected antagonistic strain was performed by the reference of Bergey's Manual of Systematic Bacteriology (20) and Laboratory Manual of General Bacteriology (21).

In vitro fungal inhibition assay

Two different techniques were used for the test of antagonistic effect against the plant root-rotting fungus *F. solani*. In the first, for assay of antifungal activity of bacterial strains on plates, samples (5μl containing approximately 10^6 cells) from overnight cultures of bacterial strains in nutrient broth (NB) were inoculated 1 cm from the edge of petri plates and allowed to soak into the agar. A small plug (about 5 mm square) of *F. solani* inoculum from the leading edge of a culture of *F. solani* grown at 28°C for 3 days on PDA containing 0.2% chitin was placed in the center of the plate. Plates incubated at 28°C and scored after 4 or 5 days by measuring the distance between the edges of the bacterial colony and fungal mycelium. Inhibition ratio was expressed relative to a control strain spotted on the sample plate. In the second, for assay of antifungal activity in broth culture, bacterial cultures were grown at 30°C for 84 hr with aeration. Cells were removed by centrifugation at 12,000 rpm for 20 min. The culture supernatants were then filtered aseptically through 0.45μm pore size membrane filter. The resulting filterates were stored at 4°C. (i) Petri plates were filled with culture filterate incorporated into molten PDA. After the plates were cooled, *F. solani* inoculum was placed on the agar surface, and the plates were incubated for 4 or 5 days. The diameter of *F. solani* colony was recorded, and inhibition ratio was calculated relative to a control without incorporated culture filterate. (ii) Culture supernatants were incorporated aseptically with a small plug taken from 2 to 3 day old culture of *F. solani* in 250 m*l* Erlenmeyer flasks containing 2.64% potato dextrose broth (PDB) and incubated on a rotary shaker at 28°C for 5 days. Fungal mycelia were collected on oven-dried preweighed weighing paper (Toyo filter paper No.2), dried at 90°C, and dry weights were determined. Inhibition ratio was expressed relative to a control (H_2O).

Preparation of lytic enzymes

For the preparation of lytic crude chitinase, 100 m*l* of chitin-peptone medium incubated aerobically on a rotary shaker at 160 rpm for 84 hr at 30°C. For the preparation of lytic crude laminarinase, 100 m*l* of laminarin-peptone medium incubated aerobically on a rotary shaker at 160 rpm for 72 hr at 30°C. The cultures were centrifuged aseptically as 12,000 rpm for 20 min at 4°C. The solid ammonium sulfate was added to the supernatant up to the concentration of 1.00 saturation. After standing overnight at 4°C, precipitate was collected by centrifugation and dissolved in a minimum volume of deionized water. The solution was then dialyzed

against deionized water for 48 hr. The dialyzed solution was used as a lytic enzyme preparation in this study.

Preparation of substrates

Chitin (from crab cells, Sigma) and laminarin (from Eigeniarborea, Tokyo Chemical Industrial Co.) were used as substrates. Colloidal chitin from crab shells was prepared by the method of Bemiller (22).

Enzyme assay

The activities of chitinase and laminarinase were determined by following the release of reducing sugar according to Somogyi-Nelson (23). One unit of chitinase (laminarinase) activity was defined as an enzyme quantity which liberated 10μg of glucose per hour. The reaction mixture of chitinase was contained 0.3 m*l* of 1 M sodium acetate buffer (pH 5.3), 0.5 m*l* of colloidal chitin at a concentration of 0.01% (dry matter), and 0.25 m*l* of enzyme solution. The reac-

antibiotics effective against the pathogen (5-8). Sup-

mixture of laminarinase was contained 0.3 m*l* of 1/15 M phosphate buffer (pH 5.5), 0.5 m*l* of 0.2% soluble laminarin, and 0.25 m*l* of enzyme solution. The reaction was carried out at 40°C for 2 hrs.

Mutagenesis and selection

The mutagenesis procedure was based on the method of Miller (24). The selected strain was mutagenized with N-methyl-nitrosoguanidine (NTG) which can induce a high frequency of mutations at doses. Approximately 10 m*l* of a 1.1×10^8 CFU/m*l* culture in the exponential growth phase was incubated with 100μg NTG per m*l* at 37°C for 30 min. The NTG-exposed cells were centrifuged at 12,000 rpm for 15 min at 25°C. The pellets were then suspended insterile 0.1 M phosphate buffer (pH 7.0). The washed pellets were resuspended in 10 m*l* of NB, and grown overnight at 30°C. The chitinase-deficient mutants were characterized by streaking the NTG-exposed cells on minimal agar plates containing collidal chitin as the sole carbon source.

Siderophore assay

Siderophore assay procedures were based on the method of Meyer and Scher (25-27). Siderophore production was determined by growing the bacteria in iron-deficient succinate minimal medium (SMM) containing 0.6% K_2HPO_4, 0.3% KH_2PO_4, 0.1% $(NH_4)_2\ SO_4$, 0.02% $MgSO_4 \cdot 7H_2O$, and 0.4% succinic acid. The pH was adjusted to 7.0 by addition of NaOH prior to sterilization. The test organisms were introduced into 250 m*l* Erlenmeyer flasks each containing 50 m*l* of medium and incubated on a rotary shaker at 30°C for 40 hr. The bacterial cells were removed by centrifugation at 12,000 rpm for 20 min. 50μl of 2 M $FeCl_3$ was added, to sample tubes of the culture supernatants (4 m*l* per tube), where as other tubes, to which no iron were added, served as the blank. Insoluble iron salts were removed by centrifugation at 3,000 rpm for 5 min. The absorbance of the supernatants were measured against water as a blank at 435 nm with spectrophotometer.

Results

Selection of strains inhibitory to fungal pathogen

For the selection of antagonist inhibitory to soilborne plant root-rotting *F. solani*, over 300 isolates of bacteria were initially obtained from ginseng root rot-suppressive soil. Among this isolates, 45 isolates have been alike in colony characteristics with one another were investigated antifungal activities to *F. solani*. Only 10 isolates produced a zone of inhibition of the *F. solani* $\geqq$15 mm on PDA. The isolate YPL-1 having the highest antifungal activity was selected among the 10 isolates and used in this study (Table 1).

Identification of the isolate

Microbiological characteristics of the selected strain (isolate YPL-1) are shown in Table 2 and an electron micrograph in Fig. 1. The strain was motile, gram negative, aerobic, rod bacteria. It showed oxidase positive, nitrate reduction positive, arginine dihydrolase negative. It hydrolysed starch and Tween 80, not produced fluorescent pigment. It could assimilate remarkably starch, maltose, glucose, L-proline, and L-aspartate, but could not L-histidine. After due consideration of morphological, culture, physiological, biochemical, and nutritional characteristics except for sorbitol utilization, refered to Bergey's Manual of Systematic Bacteriology, isolate YPL-1 was presumed to be *Pseudomonas stutzeri* or its near species.

Antifungal mechanism of *P. stutzeri* YPL-1 against *F. solani*

Table 1. Selection of antagonistic bacteria from rhizosphere in ginseng cultivated soil

Strains	Antifungal activity	
	Fungal wt. (mg)[a]	Inhibition (%)[b]
YPL-1	126.2	61.2
YBL-7	140.0	55.9
YSL-1	143.3	54.9
YSL-2	200.3	36.9
YSL-4	192.0	39.5
YML-1	164.7	49.1
YML-2	205.5	35.3
YML-3	134.1	57.8
YCL-1	276.7	13.8
YCL-2	314.5	0.1
Control	319.5	0.0

[a]Dry weights of *F. solani* with culture filterates (20% of potato dextrose broth) of selected strains were determined after 5 days incubation at 28°C.
[b]100%-relative dry weights of *F. solani* to a control (H_2O)
The all data values are the means from three replication

For the investigation of antifungal mechanism of selected *P. stutzeri* YPL-1 against *F. solani*, antifungal activities in culture filterate of the strain were compared by the following three subjects: (i) dialyzed culture filterate by cellulose dialysis sack (MW 12,000), (ii) evaporated non-protein solution of the supernatant after culture filterate treated by cold ethyl alcohol, and (iii) heat-treated culture filterate,

Table 2-A. Morphological and cultural characteristics of the isolated strain YPL-1

Characteristics	Isolate YPL-1
Cell form	Rod
Cell diameter. μm	0.7-0.8
Cell length, μm	1.5-2.7
Fragella arrangement	Polar
Number of flagella	1
Growth at 4°C	−
Growth at 41°C	±
Growth at pH 3.6	−
Need at least 12-15 NaCl for growth	−

+: Positive, −: Negative, ±: Doubtful

Table 2-B. Physiological and biochemical characteristics of the isolated strain YPL-1

Characteristics	Isolate YPL-1
Catalase test	+
Oxidase test	+
Starch hydrolysis	+
Tween 80 hydrolysis	+
Lecithinase (egg yolk)	−
Arginine dihydrolase	−
Gelatin liquefaction	−
Denitrification	+
Fluorescent pigment	−
Production of pyocyanin pigment	−
Production of pyoverdins	−

Table 2-C. Nutritional characteristics of the isolated strain YPL-1

Characteristics	Isolate YPL-1
Utilization of Succinate, Fumarate, Lactate	+
α-Ketoglutarate, Fructose, Ethanol	+
Maltose, Starch, Glucose	+ +
Glutarate, Malonate, Propionate	±
Citrate, Pyruvate, Sorbitol	+
Butanol, Isobutanol, Etylen glycol	±
L-Alanine, L-Glutamine, L-Isoleucine	+
L-Serine, L-Valine, *p*-Hydroxybenzote	±
L-Asparatate, L-Proline	+ +
L-Leucine, Glycine	+
L-Histidine	−

+ +: Good, +: Fair, ±: Doubtful, −: Not utilized
The growth was checked after 14 days culture at 30°C

at 80°C for an hour. As shown in Table 3, in consideration of the loss of 11% dialyzed inner solution, 82% non-protein solution, or 80.6% or heat-treated solution as compared with culture filterate, antagonistic substance in inhibitory mechanism of *P. stutzeri* YPL-1 to *F. solani* was presumed to be heat unstable, macromolecular substances such as protein.

To elucidate what kind of enzymes are responsible to the antifungal mechanism, chitinase production and antifungal activities against *F. solani* were investigated in the culture filterates of 8 isolates first

Fig. 1. Electron micrograph of *P. stutzeri* YPL-1. The bar denotes 1μm

Table 3. Antifungal mechanism of *P. stutzeri* YPL-1 against *F. solani*

	Antifungal activity			
	Fungal dry weight		Fungal colony size[a]	
	Inhibition (%)[b]	Relative (%)	Inhibition (%)[c]	Relative (%)
Culture filterate [A]	59.7	100.0	55.6	100.0
Dialyzed sol. [B]	53.9	89.9	48.9	87.9
Non-protein sol. [C]	11.9	19.9	8.9	16.0
Heat-treated sol. [D]	11.2	18.8	11.1	20.0

[A]The culture was grown in chitin-peptone medium at 30°C for 84 hr with *P. stutzeri* YPL-1, Chitinase production in the culture filterate = 18.2 U/m*l*.

[B]Dialyzed culture filterate, against ionized water at 4°C for 48 hr through cellulose dialysis sack (MW 12,000).

[C]Evaporated solution of supernatant after the culture filterate was precipitated by cold ethyl alcohol (up to 80%).

[D]Culture filterate was heat-treated by 80°C for 1 hr.

[a]Colony diameter (mm) of *F. solani* on culture filterate of *P. stutzeri* YPL-1 incorperated into molten PDA.

[b]100%-relative dry weight of *F. solani* to a control (H_2O).

[c]100%-relative colony size of *F. solani* to a control without incorperated culture filterate.

of all. All of the isolates showed that antifungal activities increased in proportion to chitinase production (Table 4). Also, several enzyme activities were examined in the crude enzyme preparation from the culture filterate of *P. stutzeri* YPL-1. Glucanase (46.8 unit of amylase, 43.6 unit of pullulanase, 42.9 unit of cellulase, 22.6 unit of laminarinase) besides chitinase activity was examined, and chitinase activity or β-(1,3) glucanase (laminarinase) activity was considered to participate in this lysis of 47% chitin or 14% β-1,3 glucan component of *F. solani* cell wall (8). These results indicate that the antifungal mechanism of *P. stutzeri* YPL-1 depends on lysis of *F. solani* appears to be involved in the enzymatic hydrolysis of glucan and chitin which were composed of the cell wall of fungal hyphae.

Table 4. Chitinase production and antifungal activities of the selected strains against *F. solani*

Strains	Chitinase production (U/m*l*)	Antifungal activity	
		Fungal wt.(mg)	Inhibition (%)
YPL-1	15.1	113.3	59.2
YBL-7	11.4	125.0	55.0
YSL-1	9.7	127.6	54.0
YSL-2	8.2	182.9	34.1
YSL-4	6.6	174.3	37.2
YML-1	9.9	137.5	50.5
YML-2	7.0	187.2	32.6
YML-3	6.4	124.9	55.0
H_2O	0.0	277.7	0.0

Antifungal activity by chitinase defective mutants

Since the reduced suppressiveness of chitinase defective mutants could be due to their inability to estabilish significant mechanism of lysis against *F. solani*, studies were carried out to evaluate different abilities to inhibit growth of *F. solani*. Mutants deprived of the productivity of chitinase and/or laminarinase were obtained by NTG mutagenesis. *P. stutzeri* YPL-M122 (*chi*$^-$, *lam*$^-$) was completely unable to inhibit against *F. solani*, but *P. stutzeri* YPL-M158 (*chi*$^-$) was poor (Table 5). These results confirm that antifungal mechanism of *P. stutzeri* YPL-1 for biocontrol of *F. solani* was caused by the enzymatic hydrolysis of cell wall components by more chitinase and less laminarinase.

Production of siderophore by *P. stutzeri* YPL-1

To investigate antifungal mechanism by extracellular siderophore which efficiently chelate environ-

Table 5. Antifungal activities of the chitinase negative mutants against *F. solani*

Strains	Chitinase activity (Unit)	Laminarinase activity (Unit)	Antifungal activity	
			Fungal inhibition (%)	Antag. distance[a] (%)
YPL-1	13.7	19.2	49.9	100.0
YPL-M122	0.0	0.0	0.6	0.0
YPL-M153	0.0	8.4	4.1	32.0

[a]Distance between the edges of the bacterial colony and fungal mycelium after 5 days incubation at 28°C.

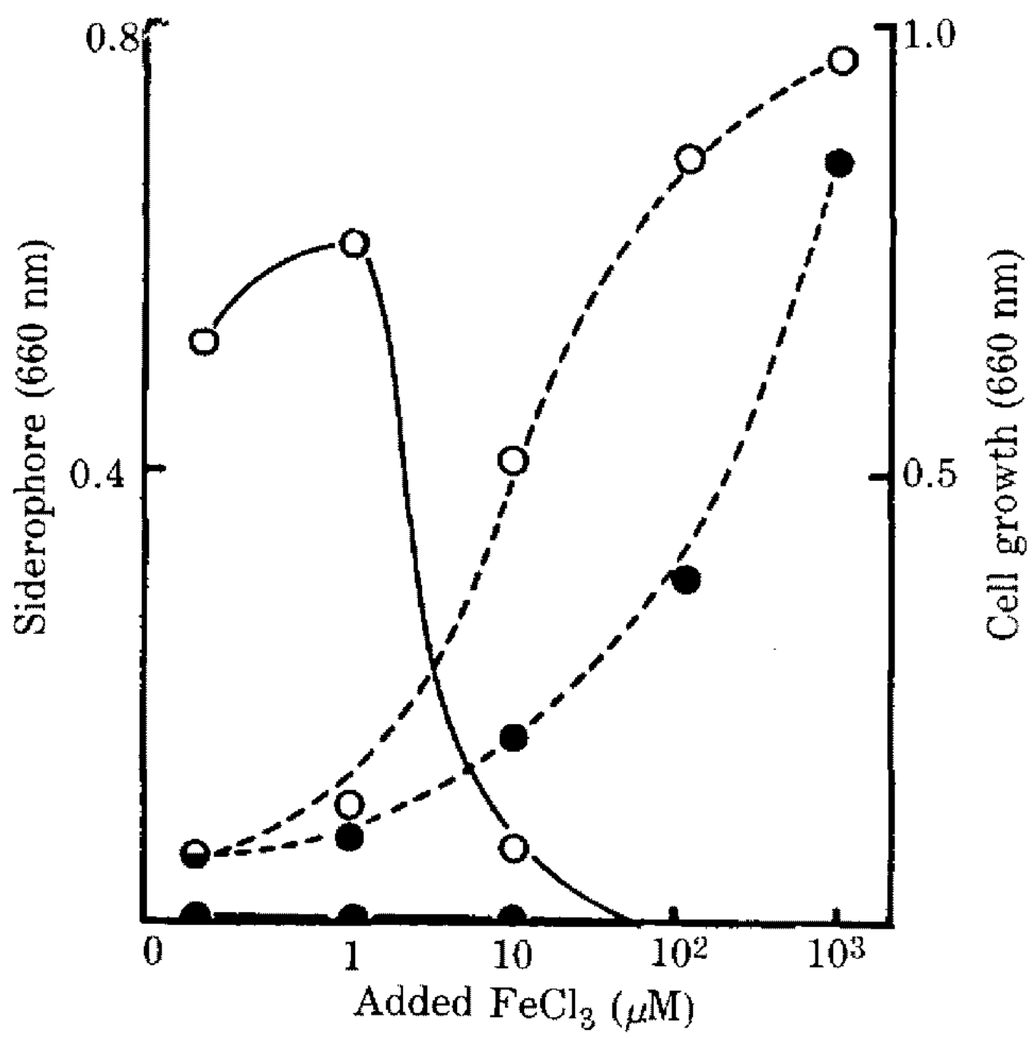

Fig. 2. Growth and siderophore production of *P. stutzeri* YPL-1
●—● siderophore of *P. stutzeri* YPL-1
●--● growth of *P. stutzeri* YPL-1
○—○ siderophore of *P. fluorescens*
○--○ growth of *P. fluorescens*

mental iron, making it less available to certain native microflora, the possibility of siderophore production by *P. stutzeri* YPL-1 was evaluated after 40 hr of growth in SMM amended with different concentrations of $FeCl_3$ as compared with siderophore-producing strain *Pseudomonas fluorescens* have been reported previously (25). In result, siderophore produced by *P. fluorescens* in the low-Fe medium was recognized by their district yellow-green pigment and fluorescent. But, *P. stutzeri* YPL-1 was not able to produce extracellular siderophore in iron-deficient medium, entirely (Fig. 2).

Discussion

Selection of antagonistic microorganisms underpins virtually all biological control programs. This procedure seems formidable given the vast numbers of individuals from which to choose in typical microbial communities. The source of antagonists should properably be from situations where naturally occurring biological control is manifest. In addition, effective antagonists from foreign habitats may also be included. A gluideline for selection and further testing of bacterial antagonists for biological control has recently been described by Andrews (28). For the selection of antagonist inhibitory to *F. solani* causing root rot of many important crops, *in vitro*, fore approaches were utilized (Table 1): (i) over 300 bacterial isolates were made on NA from ginseng root rot-suppressive soil, (ii) 45 isolates were made on chitin agar, typically based on colony and nutritional characteristics of the desired microbial component, (iii) 10 isolates, selected by the dual culture techniques on agar plates aimed at detecting inhibition zones between colonies of *F. solani* and isolates as judging by zones of inhibition of the *F. solani* ≥15 mm on PDA, and (iv) isolate YPL-1, selected by the dual culture techniques in broth culture aimed at detecting dry weights of *F. solani* growing with culture filterates of isolates as judging by inhibition ratio of the *F. solani* ≥ 60%. In result, isolate YPL-1 having the highest antifungal activity was selected, identified to be a species to *Pseudomonas stutzeri* (Table 2). Selection procedures were given some indication of the mechanism of interaction between *P. stutzeri* YPL-1 and *F. solani* (e.q., antibiosis, lysis). Evidence of mechanism of lysis may be summarized as follows (Table 3-5): (i) only 11% loss of antifungal activity in dialyzed inner solution in comparison to culture filterate of *P. stutzeri* YPL-1 (the loss of activity may led with loss of activity of lytic enzyme during dialysis), (ii) 82% loss of antifungal activity of non-protein solution in comparison to culture filterate of *P. stutzeri* YPL-1 (rest activity may led by residual alcohol in evaporated solution), (iii) 80.6% loss of antifungal activity in heat-treated solution in comparison to culture filterate of *P. stutzeri* YPL-1, (iv) 8 isolates including isolate YPL-1; increase of antifungal activities in proportion to chitinase production, (v) antifungal inability by *P. stutzeri* YPL-M122 (*chi*⁻,

lam⁻) and antifungal poor-ability by *P. stutzeri* YPL-M153 (*chi*⁻). These results indicate that antifungal mechanism of *P. stutzeri* YPL-1 for biocontrol of *F. solani* was caused by enzymatic hydrolysis of cell wall components by more chitinase and less laminarinase.

To demonstrate mechanism of nutrient competition of interaction between *P. stutzeri* YPL-1 and *F. solani*, evidence must be estabilished experimently to confirm the operation of limiting factors (carbon, nitrogen, iron) affecting pathogenesis. For example, it should be estabilished that the pathogen requires the limiting nutritional factor for germination and subsequent estabilishment for infection of the host. Only carbon and nitrogen have been establishment as essential elements in the infection process soilborne plan ant pathogens (1). The present study suggests that addition of siderophore production to lytic mechanism may be effective in biological control of *F. solani*. Unfortunately, unlike other study (26), this *P. stutzeri* YPL-1 was not produced extracellular siderophore in iron-deficient medium, entirely (Fig. 2).

Many researches in plant pathology had dealt with determination of numbers of antagonistic microorganisms in soils. We need to search out these naturally occurring biological control system, examine them in toto, pick them apart, and isolate promising components for exploitation. In doing this, however, we must learn what is involved in the interactions and what factors are responsible for fungal propagule control. The difficult part is to manipulate the soil environmental which is complex, strongly buffered biologically, and variable in its biological activity. Progress is, nevertheless, being made in understanding mycoparasitism, germination-lysis, enhanced fungistasis, suppressive soils, and management of crop residues. Progress is also being made in the use of selected biological control agents for disease suppression. Perhaps with the recent renewed enthusiasm for biological control and the increased emphasis on discovery of alternative methods to control disease by means of other the chemicals, these areas of antagonistic relationships will be emphasized and control measures utilizing them will be devised.

요 약

근채류식물의 근부원인이 되는 토양유래의 식물병원성진균에 대한 생물학적 방제를 위하여 저병해인삼경작지토양으로부터 식물근부균 *Fusarium solani*의 생육을 강력히 길항하는 억제세균 YPL-1을 분리, 선발하였으며 이들 동정한 결과 *Pseudomonas stutzeri*이거나 그 근연종으로 확인하였다. 선발된 *P. stutzeri* YPL-1에 의해 생산된 근부균생육억제물질은 열에 민감한 고분자의 단백성물질로서 chitinase 및 laminarinase 등 *F. solani*의 외막가수분해효소인 것으로 추정된다. 더욱이 chitinase 생산능과 근부균생육억제능은 정관계로 비례한다는 것도 알았다. 이는 NTG를 이용하여 얻은 chitinase 및 laminarinase 생산불능변이주 *P. stutzeri* YPL-M122(*chi*⁻, *lam*⁻), *P. stutzeri* YPL-M153(*chi*⁻)에 의해서도 확인되었다. 그러나 본 *P. stutzeri* YPL-1은 siderophore를 전혀 생산하지는 못하였다. 이 결과로 미루어 보아 선발된 억제균 *P. stutzeri* YPL-1 균주에 의한 식물근부균 *F. solani*의 생육억제기작은 저분자물질인 항생물질이나 siderophore가 아닌 chitinase를 주로 하는 외막가수분해효소에 의한 근부균 *F. solani*의 세포벽분해에 기인된 것으로 생각된다.

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References

1. Baker, R.: *Annu. Rev. Phytopathol.*, **6**, 263 (1968).
2. Blakeman, J.P. and N.J. Fokkema: *Annu. Rev. Phytopathol.*, **20**, 167 (1982).
3. Cook, R.J.: *Phytopathol.*, **75**, 25 (1985).
4. Benson, D.M. and R. Baker: *Phytopathol.*, **64**, 38 (1974).
5. Howell, C.R. and R.D. Stipanovic: *Phytopathol.*, **69**, 480 (1979).
6. Howell, C.R. and R.D. Stipanovic: *Phytopathol.*, **70**, 712 (1980).
7. Rothrock, C.S. and D. Gottlieb: *Can. J. Microbiol.*, **30**, 1440 (1984).
8. Thomashow, L.S. and D.M. Weller: *J. Bacteriol.*, **170**, 3499 (1988).
9. Schroth, M.N. and J.G. Hancock: *Science*, **216**, 1376 (1982).
10. Kloepper, J.W., J. Leong, M. Teintze and M.N. Schroth: *Nature* (London), **286**, 885 (1980).
11. Elad, Y. and R. Baker: *Phytopathol.*, **75**, 1047 (1985).
12. Davison, J.: *Biotechnology*, **6**, 282 (1988).

13. Skujins, J.J., H.J. Potgieter and M. Alexander: *Arch. Biochem. Biophys.*, **111**, 358 (1965).
14. Morrissey, R.F., E.P. Dugan and J.S. Koths: *Soil. Biol. Biochem.*, **8**, 23 (1976).
15. Lifshitz, R., M.Y. Windham and R. Baker: *Ecology and Epidemiology*, **76**, 720 (1986).
16. Ordentlich, A., Y. Elad and I. Chet: *Phytopathol.*, **78**, 84 (1988).
17. Mitchell, R.: *Phytopathol.*, **53**, 1068 (1963).
18. Guy, S.O. and R. Baker: *Phytopathol.*, **67**, 72 (1977).
19. Henis, Y., B. Sneh and J. Katran: *Can. J. Microbiol.*, **13**, 643 (1967).
20. Krieg, N.R. and J.C. Holt: Bergey's Manual of Systematic Bacteriology, Vol.1, Williams and Wilkins Co., Baltimore (1984).
21. Gerhardt, P.: Manual of Methods for General Bacteriology, Am. Soci. Microbiol., Washington (1980).
22. Bemiller, J.N.: Method in Carbohydrate Chem., Vol.5, Academic Press, p.103 (1965).
23. Nelson, N.: *J. Biol. Chem.*, **153**, 375 (1944).
24. Jeffrey, H.M.: Experiments in Molecular Genetics, Cold Spring Harbor, Laboratory, p.125 (1974).
25. Meyer, J.M. and M.A. Abdallah: *J. Gen. Microbiol.*, **107**, 319 (1978).
26. Meyer, J.M. and M.A. Abdallah: *J. Gen. Microbiol.*, **118**, 125 (1980).
27. Scher, F.M. and R. Baker: *Phytopathol.*, **72**, 1567 (1982).
28. Andrews, J.B.: Biological Control on the Phylloplane, Edited by Carol E. Windels and Stenven E. Lindow, The American Phytopathological Society, 31 (1985).

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