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Effect of Siderophore on Biological Control of Plant Pathogens and Promotion of Plant Growth by *Pseudomonas fluorescens* ps88

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Abstract : *Pseudomonas fluorescens* ps88 was isolated from the rhizosphere soil produced the secondary metabolite called siderophore under iron limited conditions. On iron limiting KMB medium this strain inhibited the growth of *Pythium ultimum, Pyricularia oryzae, Rhizoctonia solani and Xanthomonas oryzae*. Cucumber seeds were coated with the strain ps88 and were grown in green house soil. Forty days after the seed emergence, disease incidence caused by *Fusarium oxysporium* was reduced up to 50%. When the cucumber plants were grown in vermiculite, a significant fresh weight was increased. Root development of red pepper plants was also enhanced on MS medium supplemented with siderophore(Received September 13, 1995; accepted January 24, 1996).

Introduction

Certain microorganisms associated with the roots of plants provide biological control of disease caused by soilborn pathogens.[1] The mechanisms that have been implicated in the control of soilborne pathogens by fluorescent pseudomonads are production of antibiotics, siderophores, and competition for nutrients or preferred colonization sites on the root.[2]

For example, *Pseudomonas fluorescens* 2-79 suppressed take-all, a major root disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*.[3,4] The bacterium produced an antibiotic, phenazine-1-carboxylic acid(PCA), and a fluorescent pyoverdin siderophore. Other *P. fluorescens* 3551 and HV37a controlled damping-off of cotton seedling caused by *Pythium* spp. In the former strain siderophore production was involved in disease suppression, but in the latter strain the antibiotic oomycin-A was more important.[5-7] There are other fluorescent rhizopseudomonas which do not produce antibiotic compound but promote plant growth.[8,9] Kloepper *et al*[10,11] reported that plant growth promoting rhizobacteria rapidly colonized plant roots of potato, sugarbeet and radish, and caused significant yield increase up to 144% in field test.

To elucidate the role of siderophore in plant growth enhancement, Hofte *et al* constructed mutants unable to synthesize siderophores via *Tn*5 mutagenesis. The inoculation of siderophore producing rhizopseudomonas resulted in increase of plant dry weight while inoculation of the *Tn*5 mutants did not. This result suggested that in a specific rhizosphere conditions the inoculated bacteria could produce siderophores which might be responsible for the growth enhancement. We describe effect of siderophore on biological control of some plant pathogen and promotion of plant growth by *P. fluorescens* ps88.

Key words : Siderophore, pathogens, growth promotion
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Materials and Methods

Microorganisms and culture conditions

Pseudomonas spp. isolated from the rhizosphere soil was obtained from C. S. Park, Department of Plant Pathology, Gyeongsang National University, Korea. Microorganisms were maintained for long-term storage at −80 ℃ on potato-dextrose agar(PDA) medium and on King's medium B (KMB) covered with 50% glycerol. For the experimental use, the cultures were transferred to PDA or KMB agar plate and incubated at 27℃.

Purification of siderophore produced by *P. fluorescens* ps88

The bacteria were grown in KMB broth for 48 hours and filtered after the supernatant was adjusted to pH 6 with HCl. The filtered supernatant was passed through Dowex AG-1X2 that was equilibrated with 1M NH_4Cl. The filtrate was acidified to pH 2, saturated with ammonium sulfate and extracted with benzyl alcohol. The organic layer was diluted four times with diethyl ether and was extracted three times with H_2O. The aqueous extract was washed with ethyl ether, rotary evaporated and then lyophilized.[12]

Thin layer chromatography

The purified siderophore product was dissolved in 0.2 M acetic acid-pyridine buffer (pH 4.8) and was separated by thin layer chromatography on the cellulose plate. The solvent system was butanol solution saturated with 1.7% ammonium acetate solution. The fluorescence of siderophore was visualized under UV light and the plate sprayed with ferric chloride solution (0.1%) was heated until the color developed. The chromatographic spots were scraped and dissolved in ethanol. The UV spectrum of the purified siderophore was measured using spectrophotometer (DU 70 UV/VIS, Beckman).

Biological control of pathogens *in vitro*

P. fluorescence was used to test inhibitory effect against *R. solani, P. ultimum* and *P. oryzae* on PDA. *P. fluorescens* and other strains cultured in rich broth culture were spotted around the perimeter, and a 5 mm-diameter plug of *R. solani, P. ultimum, P. oryzae* was placed at the center of each plate. The plates were incubated at 27℃ until the the fungi reached the edge of bacteria. To test the antibiotic activity of the strain *P. fluorescens* ps88, cell suspension was spotted at the center with 10 μl of cell suspension (10^6 CFU/ml) on the KMB agar plates with or without 10 mM $FeCl_3$ and was incubated 24 hr at 27℃. Cell suspension of *Xanthomonas oryzae* at 27℃ for 24 hrs (10^6 CFU/ml) was sprayed over the KMB agar plate with or wothout $FeCl_3$ and was incubated for additional 24 hrs.

Plant growth promoting effect of *P. fluorescens* ps 88

To determine the effect of siderophore on the late plant growth, pepper seedlings were grown aseptically in 400 ml glass bottles and explants were obtained. The pepper plantlets were regenerated in MS medium containing hormones at 25℃ for 45 days. The strain ps88 of *P. fluorescens* was inoculated into KMB and the siderophore production was measured by filtering through 0.2 μm filter. The partially purified supernatant was added to the pepper seedlings.

Enhenced cucumber plant growth by *P. fluorescens* ps88 in green house

The seeds treated with *P. fluorescens* were planted in vermiculite and incubated in a growth chamber at 23℃ with a 12 hr light-dark cycle. Plants were harvested 10 day after emergence. Each experiment had three treatment: seed treated with *P. fluorescence*; seed treated with sterile water only; and seed treated with *P. fluorescence* containing $FeCl_3$. The weight of total plants was measured 10 days after sowing.

Results and Discussion

Purification of siderophore

The purified siderophore was dissolved with ethanol and its UV spectrum was measured. A typical spectrum of siderophore at 400 nm appeared when *P. fluorescence* ps88 was grown in iron depleted medium (Fig. 1-A). When the strain ps88 grown in iron rich medium little siderophore was produced (Fig. 1-B), resulting in small absorption peak at 400 nm. The common peak of siderophores produced by different species of pseudomonads appeared at 400 nm. This peak disappeared when the cell were grown in iron rich medium. Barghouthi *et al*[13] showed that the phenolate siderophore produced by *Aeromonas hydrophila* appeared at 314 nm. Therefore, this result suggest that siderophore was produced by *P. fluorescens* ps88.

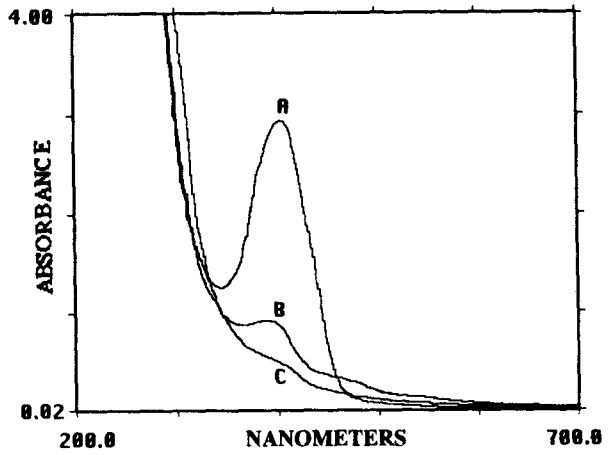


Fig.1. UV adsorption spectra of siderophores produced by *Pseudomonas fluorescens* ps88 grown in iron depleted KMB medium (A), the strain of Pseudomonas fluorescens ps88 grown in KMB^+ $FeCl_3$ (B) and *E. coli* HB101 grown in KMB medium (C).

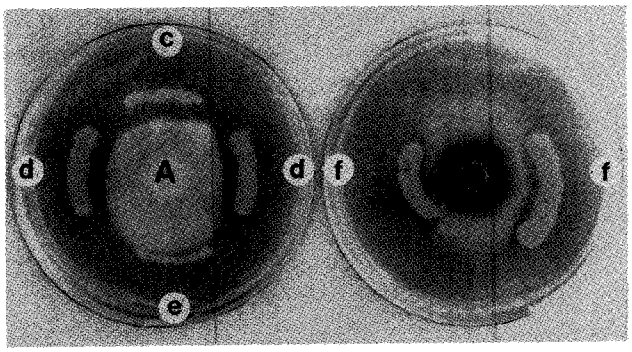
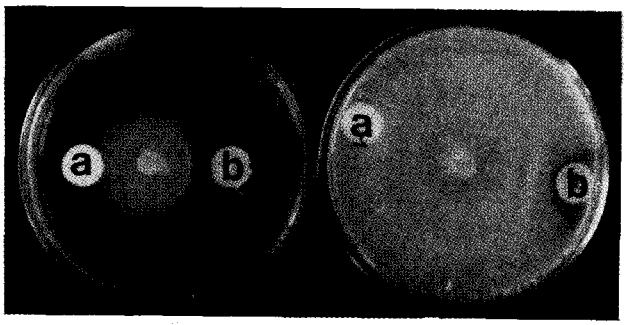


Fig. 2. Antifungal activity of *Pseudomonas fluorescens* ps88 against *Pythium ultimum* (A) and *Pyricularia oryzae* (B) on KMB medim. c, *Pseudomonas* spp.; d, f, P. *fluoresecens* sp 88; e, *E. coli* HB101

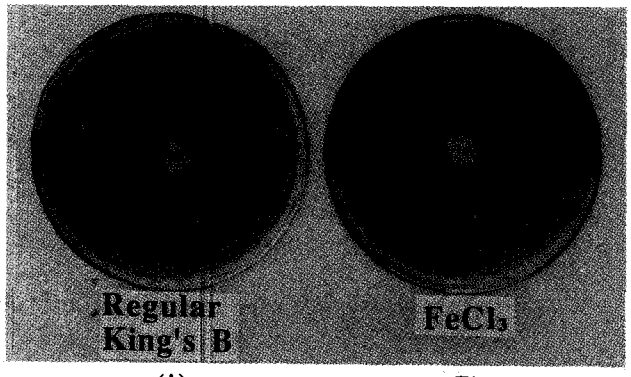
Biological control of plant pathogens by *P. fluorescence* ps88

To examine an antifungal activity of siderophores produced by the strain ps88, *P. ultimum* and *P. oryzae* were grown on the iron limiting KBM. On KBM, the strain ps88 inhibited the growth of *P. ultimum* (Fig. 2-A) and *P. oryzae* (Fig. 2-B). The purified siderophore from the TLC plate was scrapped and was used to bioassay against *P. ultimum* (Fig. 3-A) and *R. solani* (Fig. 3-B). The antifungal activities of the purified siderophore against *P. ultimum* and *R. solani* were defined that it was due to compound substance with antibiotics or siderophore itself. In general, the antifungal effect or growth inhibition of siderophore against pathogen was reported to be due to strong iron chelating capacity.[14] On the other hand, the majority of antifungal activity reported was due to the production of antibiotic compounds.[15,16] But in our experiment, the purified siderophore exhibited similar inhibitory effects on pathogen. Fig. 4 shows the effect of siderophore production in control of *X. oryzae* on KBM where the inhibition zone was greater on the iron depleted KMB medium. The inhibition zone was reduced on the KMB supplemented with 10 mM iron, indicating that the strain ps88 may produce some antifungal substances both in the presence or absence of iron, however.



(A) (B)

Fig. 3. Inhibition assay of siderophore extracted from TLC plate against *P. ultimum* (A) and *R. solani* (B) on KMB medium. a, Control (ethanol); b, Purified siderophore



(A) (B)

Fig. 4. Control of *Xanthomonas oryzae* by *Pseudomonas fluorescens* ps88 on regular King's B medium(A) and King's B medium supplemented $FeCl_3$(B).

Disease incidence

Effect of agent ps88 on the biological control of *Fusarium oxysporium* wilt was examined in the green house using cucumber plant for 80 days. The *Fusarium* wilt appeared at about two weeks after transplanting. After 60 days in control soil, disease incidence by Fusarium reached to 50%, whereas in the soil inoculated with the strain ps88 the disease incidence was only 16%. After 80 days, almost 70% plants in control soil was infected by Fusarium wilt and and 30% was infected in the soil inoculated with the strain ps88.

Plant growth promoting effect

When the cucumber seeds were soaked with the strain ps88, the growth of cucumber was enhanced significantly (Table 1). Also the enhancement of hairy root development in the MS medium supplemented with si-

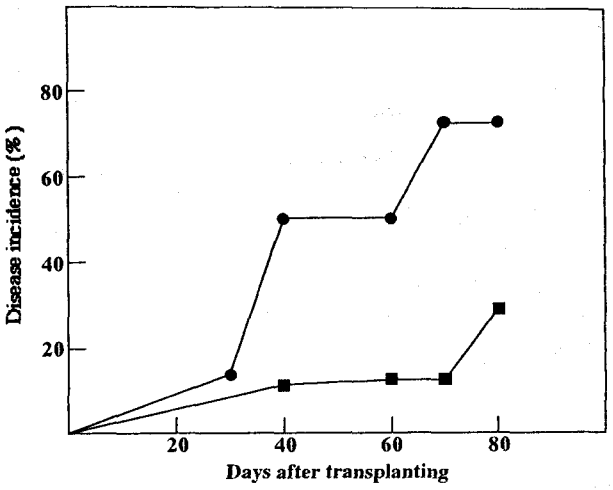
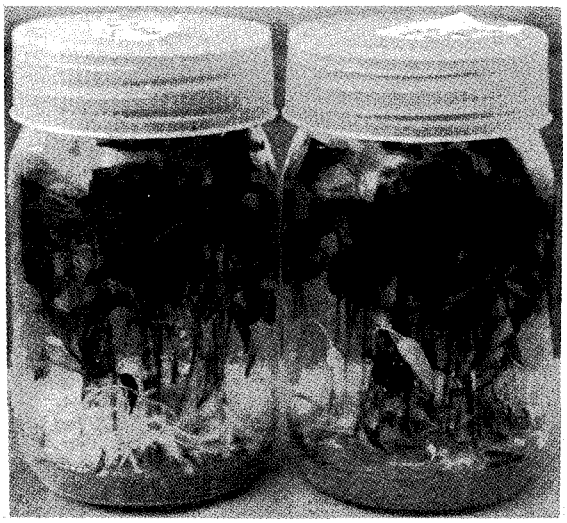


Fig. 5. Disease incidence of cucumber wilt by *Fusarium oxysporium* in vinylhouse soil when the plants were inoculated with the biocontrol agent, *Pseudomonas fluorescens* ps88. ●—●, Control; ■—■, *Pseudomonas fluorescens* ps88.

Table 1. Enhanced cucumber plant growth by *Pseudomonas fluorescens* ps88 in green house

Treatment	Average plant weight (g)
Control (H_2O)	1.0
P. fluorescens ps88	2.1*
P. fluorescens ps88+$FeCl_3$	1.3

Cucumber seeds were planted in vermiculate. Cucumber seed were dipped in cell suspension (10^9 CFU/ml). Pots were watered with 120 ml of nutrient solution every two days. Plants were harvested 10 days after seed germination. The average of 6 replicates with three plants per pot was used for plant weight. *Statistically significant at $p=0.01$.



(A) (B)

Fig. 6. Effect of *Pseudomonas fluorescens* ps88 on plant growth. A: Pepper plants grown in MS medium supplemented with filtrated siderophore produced by *Pseudomonas fluorescens* ps 88, B: Pepper plants grown in MS medium supplemented without siderophore.

derophore was observed (Fig. 6). The purified siderophore produced by strain ps88 might be inpart responsible for fungal inhibition *in vitro* under iron depleted conditions. According to Hamdan *et al*, the fluorescent siderophores and the other factors are involved in biological control of *Gaeumannomyces graminis* by *P. fluorescens* 2-79 and M4-80R suggesting that the mechanism of action of fluorescent growth-promoting Pseudomonads may be invoved with antigonism to minor pathgens. Also, growth responses induced by *Trichoderma* spp. appear to be due to both the control of minor pathogens and production of a growth-regulating factor.[17] Our result of mimic antifungal activities by *P. fluorescens* in iron supplemented KMB medium and enhencement of root devolpment on MS medium supplemented with siderophore may be siderophore itself or associated unknown growth-regulaing factor may cause a synergistic effect in plant growth.

References

1. Leong, S. A. (1989) Siderophores in plant-pathogen interactions, p.62-83. In Kosuge, T. and E.W. Nester (ed.), Plant-microbe interactions, Vol. 3. McGraw-Hill publishing Company, New York.
2. Baker, J. O. and R. J. Cook (1988) Role of siderophores in suppression of Pythium species and production of increased-growth response of wheat by fluorescent pseudomonads. *Phytopathol.* **78**, 778-782.
3. Bull, C. T., D. M. Weller, and L. S. Thomashow (1991) Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici by Pseudomonas fluorescens* strain 2-79. *Phytopathol.* **81**, 954-959.
4. Hamdan, H., D. M. Weller, and L. S. Thomashow (1991) Relative importance of fluorescent siderophores and other factors in biological control of *Gaeumannomyces graminis* var. *tritici by Pseudomonas fluorescens* 2-79 and M4-80R. *Appl. Environ. Microbiol.* **57**, 3270-3277.
5. Di Pietro, A., M. Gut-Rella, J. P. pachlatko, and F. J. Schwinn (1992) Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum*, a causal agent of damping-off. *Phytopathol.* **82**, 131-135.
6. Kraus, J. and J. E. Loper (1992) Lack of evidence for a role of antifungal metabolite production by *Pseudomonas fluorescens* Pf-5 in biological control of *Pythium damping-off of cucumber. Phytopathol.* **82**, 264-271.
7. Loper, J. E. (1988) Role of fluorescent siderophore production in biological control of *Pythium ultimum* by a *Pseudomonas fluorescens* strain. *Phytopathol.* **78**, 166-172.
8. Hofte, M., M. Mergeay, and W. Verstraete (1990) Marking the *Rhizopseudomonas* strain 7NSK2 with a Mu *d(lac)* element for ecological studies. *Appl. Environ. Microbiol.* **56**, 1046-1052.
9. Seong, K. Y., M. H fte, and W. Verstraete (1992) Acclimatization of plant growth promoting Pseudomonas strain 7NSK 2 in soil: Effect on population dynamics and plant growth. *Soil Biol. Biochem.* **24**, 751-759.
10. Kloepper, J. W. and M. N. Schroth (1981) Relationship of *in vitro* antibiosis of plant-promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathol.* **71**, 1020-1024.
11. Kloepper, J. W., J. Leong, M. Teintze, and M. N. Schroth (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**, 885-886.
12. Schwyn, B. and J. B. Neiland (1987) Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **160**, 47-56.
13. Barghouthi, S., R. Young, M. O. J. Olson, J. E. L. Arceneaux, L. W. Clen, and B. R. Byers (1989) Amonabactin, a novel tryptophan- or phenylalanine-containing phenolate siderophore in *Aeromonas hydrophila. J. bacteriol.* **171**, 1811-1816.
14. Buyer, J. S. and J. Leong (1986) Iron transport-mediated antagonism between plant growth-promoting and plant-deleterious *Pseudomonas strains. J. Biol. Chem.* **261**, 791-794.
15. Howie, W. J. and T. V. Suslow (1991) Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in the cotton spermosphere and rhizosphere by *Pseudomonas. Mol. plant-Microbe Interactions* **4**, 393-399.
16. Maurhofer, M., C. Keel, U. Schnider, C. Voisard, D. Haas, and G. Defago (1992) Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHA0 on its disease suppressive capacity. *Pyhtopathol.* **82**, 190-195.
17. Baker, R. 1988. Trichoderma spp. as plant-growth stimulants. *CRC critical reviews in biotechnology.* **7**, 97-106.

Pseudomonas fluorescens ps88이 생성하는 siderophore가 병원균의 생물학적 방제와 식물생육에 미치는 영향

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초록 : 근권토양에서 분리한 형광성 *P. fluorescens* ps88은 철이 결핍된 환경에서 2차대사산물인 siderophore라는 형광성물질을 분비하며, KMB배지에서 *Pythium ultimum, Pyricularia oryzae, Rhizoctonia solani* 및 *Xanthomonas oryzae*의 생육을 억제시켰다. 오이의 씨앗을 *P. fluorescens* ps88로 접종한 후 *Fusarium*의 발병율을 조사하였을 때 ps88 접종구에서는 이병율이 대조구에 비하여 50%가 감소하였다. Vermiculate에서 균을 접종한 후 오이의 생육촉진효과를 조사한 결과 대조구보다 현저한 생육증가가 관찰되었으며, MS배지에서 고추의 뿌리에 siderophore를 처리하였을 때 대조구에 비하여 미세한 뿌리의 발달이 관찰되었다.

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