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Root Colonizing and Biocontrol Competency of *Serratia plymuthica* A21-4 against Phytophthora Blight of Pepper

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The biocontrol agent *Serratia plymuthica* A21-4 readily colonized on the root of pepper plant and the bacterium moves to newly emerging roots continuously. The colonization of A21-4 on the pepper root was influenced by the presence of *Phytophthora capsici* in the soil. When *P. capsici* was introduced in advance, the population density of A21-4 on the root of pepper plant was sustained more than 10^6 cfu/g root until 3 weeks after transplanting. On the other hand, in the absence of *P. capsici*, the population density of A21-4 was reduced continuously and less than 10^5 cfu/g root at 21 days after transplanting. *S. plymuthica* A21-4 inhibited successfully the *P. capsici* population in pepper root and rhizosphere soil. In the rhizosphere soil, the population density of *P. capsici* was not increased more than original inoculum density when A21-4 was treated, but it increased rapidly in non-treated control. Similarly, the population density of *P. capsici* sharply increased in the non-treated control, however the population of *P. capsici* in A21-4 treated plant was not increased in pepper roots. The incidence of Phytophthora blight on pepper treated with A21-4 was 12.6%, while that of non-treated pepper was 74.5% in GSNU experimental farm experiment. And in farmer's vinyl house experiment, the incidence of the disease treated with the fungicide was 27.3%, but treatment of A21-4 resulted in only 4.7% of the disease incidence, showing above 80% disease control efficacy.

Keywords : biological control, pepper, phytophthora blight, root colonization, *Serratia plymuthica* A21-4

Phytophthora blight of pepper is the most common and severe in pepper cultivation both in open field and the greenhouse. Oospore, chlamydospore and resting hyphae are known to survive in soils as primary inoculum sources, however, most of the infections and rapid spread of the disease mainly caused by zoosporangia and zoopsores. Prolonged rain or improper irrigation in poor drainage soil leads excessive wet conditions which favor the disease epidemics. The zoospores of *Phytophthora capsici* in rhizosphere soil vividly move through soil water and make new infections. The amount of zoospores in soil at the beginning of pepper cultivation is directly related to the amount of disease outbreak during the growing season (Schlub et al., 1983). Although numerous attempts have been made to control the disease biologically, most of them were not practically feasible. Main reason is the population density of antagonists was not sufficient to suppress the pathogen throughout the growing season. A biocontrol agent, *Serratia plymuthica* A21-4 was selected from the roots of onion and A21-4 significantly inhibited the mycelial growth, zoosporangia formation and cystospore germination of *P. capsici in vitro* (Shen et al., 2002). In this study, we investigated a root colonizing ability of the biocontrol agent *S. plymuthica* A21-4, its influence on population changes of *P. capsici*, and efficiency on control of Phytophthora blight of pepper in fields.

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Material and Methods

Antagonistic strain and pathogen preparation. *Serratia plymuthica* strain A21-4 was grown at 28°C in tryptic soy broth (TSB) and stored at -70°C in TSB containing 20% glycerol. The strain A21-1 was marked with rifampicin resistance for the recovery. *Phytophthora capsici* Pa-61 (KACC 40476) was maintained on V8 juice agar and its zoospores collected from V8 juice agar were used as inocular to induce the disease.

Analysis of population density of A21-4 and *P. capsici* in soil and pepper root. The population density of A21-4 was determined by dilution plate on 1/10 strength of TSA containing 50 μl/ml rifampicin. Colony forming units (cfu) of *P. capsici* in soil or pepper roots were enumerated using corn-meal agar with supplement of antibiotics (Pimaricin 0.4 ml, Rifampicin 10 mg, Ampicillin 300 mg, Hymexazole 150 mg, PCNB 300 mg per 1000 ml DW).

Evaluation of disease suppression by A21-4

Pot experiment: The roots of 50-days-old pepper seedlings (variety Nok-Kwang) obtained from the commercial plug nursery were soaked in the bacterial suspension of A21-4 (10^9 cfu/ml) for one hour and transplanted to the pots. Pot

mix soil (Tosilee Sinangro Co., Korea) was previously infested with zoospore suspension of *P. capsici*. Ninety gram of pot mix soil was infested with 10 ml of zoospore suspension (10^4 spores/ml) of *P. capsici*. After transplanting, population density of A21-4 and colony forming units of *P. capsici* in soil or pepper roots were enumerated, and the infected plants showing typical Phytophthora blight symptom were carefully examined.

Field experiment: GSNU experiment: The roots of 50-day-old pepper seedlings (variety Nok-Kwang) were soaked in the bacterial suspension (10^9 cfu/ml) for one hour and transplanted to the green house. Experiment plot was arranged in randomized block design with four replicates, 26 plants per plot. The plants were cultivated in the greenhouse using common farming practices.

Farmer's vinyl house experiment: A farmer's vinyl house was located in Daegok-Myon, Jinju-Si, where pepper has been continuously cultivated and Phytophthora blight occurred severely in previous years. The roots of 50-day-old pepper seedlings (variety Nok-Kwang) which grown in commercial plug nursery were soaked in the bacterial suspension (10^8 cfu/ml) for one hour and transplanted to the vinyl house. Seven days after transplanting, A21-4 suspension (10^8 cfu/ml) was treated again by drenching into the pepper rhizosphere soils. The disease suppression by A21-4 was compared to chemical fungicide treatment (ethaboxam: Guardian Bayer CropScience Co. Korea, Spraying four times intervals of 10 days with 1000 ×). The numbers of infected plants were examined afterward. The method and conditions of pepper cultivation in the vinyl house was followed by ordinary farming practices in Jinju area.

Results

Population change of A21-4 in pepper growing soil and pepper roots. Pepper seedlings were soaked into the bacterial suspension and transplanted to the pot. The population densities of A21-4 maintained over 10^6 cfu/g in the inoculated original root, and moved to the newly emerging roots and effectively colonized in the surrounding soil over 10^6 cfu/g soil (Table 1). In the rhizosphere soil, the population densities of A21-4 maintained over 10^6 cfu/g soil in all of the treatments until 21days after treatment. When the pepper plants inoculated with A21-4 were transplanted to the soil infested with zoospores of *P. capsici*, the density of A21-4 was decreased a little until 14days after transplanting, however the density was recovered 21 days after transplanting. But when the plants were transplanted to pathogen free soil, the density of A21-4 decreased slightly up to 21 days after transplanting (Table 2).

Table 1. Colonization of *Serratia plymuthica* A21-4 on the pepper root and soil when the bacterium was introduced by soaking the pepper roots in the suspension of A21-4 cell before transplanting

Sampling site	Population densities after transplanting (Log cfu/g)		
	7 days	14 days	21 days
Original root	6.5 b[a]	6.2 b	6.1 a
Newly emerging root	6.4 b	6.1 b	5.7 ab
Rhizospere soil	7.3 a	7.0 a	6.1 a

[a] Different letter in the column meant significantly different at 5% probability level (Turkey's studentized range test).

Table 2. Influence of pathogen *Phytophthora capsici* on the colonization of antagonist *Serratia plymuthica* A21-4 in pepper root and soil

Sampling site	*P. capsici*	Population density (Log cfu/g)		
		7 days	14 days	21 days
Pepper root	Presence	6.6 a[a]	6.1 a	6.7 a
	Absence	6.9 a	6.2 a	5.6 b
Rhizosphere soil	Presence	7.3 a	7.0 a	6.1 a
	Absence	7.5 a	7.2 a	6.4 a

[a] Different letter in the column meant significantly different at 5% probability level (Turkey's studentized range test).

Suppression of population density of *P. capsici* in rhizosphere soil and pepper root by A21-4. The roots of 50-day-old pepper seedlings were soaked in the bacterial suspension and transplanted to the *P. capsici* infested soil. In the rhizosphere soil, the population density of *P. capsici* did not increase more than original inoculum density throughout the experimental period in A21-4 treated. while the population density of *P. capsici* was increased rapidly and reached to 5 times of original inoculum density at 21days after treatment in non-treated control (Fig. 1). In pepper roots, the population density of *P. capsici* increased greatly in the non-treated control, however the population of *P. capsici* in A21-4 treated plant did not increase (Fig. 1).

Biological control of Phytophthora blight of pepper by *Serratia plymuthica* A21-4

Disease suppression in GSNU experimental farm: An experiment for the suppression of Phytophthora blight by A21-4 was carried out in a vinyl-house located at the Gyeongsang National University experimental farm in 2001. The pepper seedlings soaked in the suspension of A21-4 and untreated seedlings were transplanted in the vinyl-house soil that was previously infested with *P. capsici*. One month after transplanting, the incidence of Phytophthora blight was 74.5% in non-treated control, while it was only 12.6% in A21-4 treatment. The disease incidence of treated and non-treated plots were sustained until end of the crop season (Table 3). Pepper yields in A21-4 treated plant

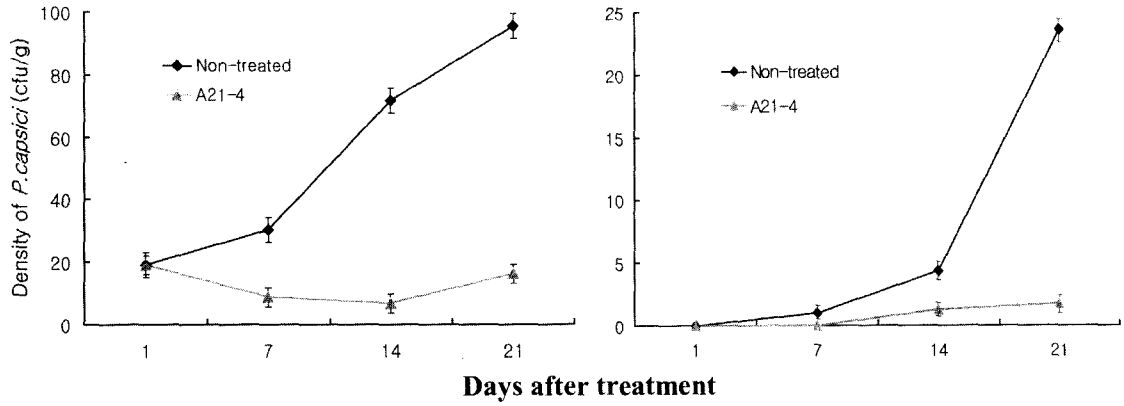

Fig. 1. Inhibitory effect of *Serratia plymuthica* A21-4 on the population densities of *Phytophthora capsici* in the rhizosphere soil (left) and on pepper root (right).

increased 141.8% compare to non-treated plants (Table 5).

Disease suppression in farmer's vinyl house: An experiments for the control of Phytophthora blight of pepper with *Serratia plymuthica* A21-4 was carried out in a farmer's vinyl house located at Daegok-Myon in Jinju city in 2002. The pepper seedlings were soaked in the A21-4 suspension and transplanted in the vinyl house field. Seven days after transplanting, A21-4 suspension was treated again by drenching into the pepper rhizosphere soils. The disease incidence of pepper plants treated with a fungicide, ethaboxam (Guardian Bayer CropScience Co. Korea) were compared. The cultural practices of pepper in vinyl house was followed by ordinary farming practices in Jinju area.

One month after transplanting, the infection rate of pepper treated with ethaboxam was 5%, while no diseased plant was observed in the A21-4 treated plants. Five months after transplanting, the infection rate of pepper treated with the fungicide was 27.3%, however, that of A21-4 treated pepper was only 4.7% (Table 4, Fig. 2). Unfortunately, although there was a great difference in pepper yields between the two plots, it was not compared in this experiment.

The field experiment was repeated in 2003, however,

Table 3. Suppression of Phytophthora blight of pepper in a greenhouse by *Serratia plymuthica* A21-4 when the root of pepper seedlings were soak in the bacterial suspension before transplanting

Treatment	Disease incidence[a] (%)
A21-4	12.6 a[b]
Untreated	74.5 b

[a] The disease incidence was observed on 60 days after treatment.
[b] Different letter in the column means significantly different at 5% probability level (Turkey's studentized range test).

Table 4. Effect of *Serratia plymuthica* A21-4 on suppression of Phytophthora blight in a farmer's vinyl house compare with a fungicide

Treatment	Disease incidence (%)		
	30 days	60 days	150 days
A21-4	0.0 a[a]	2.3 a	4.7 a
Chemicals	5.0 b	13.7 b	27.3 b

[a] Different letter in the column means significantly different at 5% probability level (Turkey's studentized range test).

Fig. 2. An overview of a farmer's pepper experimental field. Plants in the right row treated with *Serratia plymuthica* A21-4 grow healthy and the plants in the left row treated with a fungicide, ethaboxam treated show severe symptoms after 150 days after transplanting.

Table 5. Accumulated yield of green pepper fruits harvesting from healthy looking plants that either treated with A21-4 or none in GSNU experimental farm

Treatment	Pepper yield (g/10 plant)	Increasing rate (%)
A21-4	4907.6 a[a]	141.8
Control	3461.5 b	100.0

[a] Different letter in the column means significantly different at 5% probability level (Turkey's studentized range test).

typical symptoms of the Phytophthora blight of pepper were not induced due to infavorable environmental conditions. However, the growth of pepper seedlings treated with A21-4 was superior to non-treated plot.

Discussion

Suppression of Phytophthora blight of pepper is inevitably related with suppression of zoosporangia production and germination of zoospores. In previous researches conducted by the authors, *S. plymuthica* strain A21-4 successfully inhibited the germination of zoosporangia and cystospores, and also formation of zoosporangia and zoospore of *P. capsici in vitro* (Shen et al., 2002). It is presumed that A21-4 principally suppresses the Phytophthora population by inhibition of zoospores germination and zoosporangia formation on the root and in the rhizosphere of pepper.

The most important factor for the success of biological control of soil borne diseases is how to maintain the population density of biocontrol agent sufficently for prolonged time in the rhizosphere and on root system. Many works reported to maintain the population density of biocontrol agent by formulation of biocontrol agent (Hur et al., 1990; Park et al., 1989) or amendment with organic materials (Kim, 1995; Nam et al., 1988). But it is not enough to maintain sufficient the population in root or rhizosphere soil when microorganism introduced in to soil or root system. Therefore, excellent root colonization is inevitably necessary for the suppression of root pathogens (Kaiser et al., 1989; Kloepper et al., 1991; Schippers et al., 1987). Bacteria growing in or near the infection courts of roots are ideally positioned to inhibit root pathogens early in pathogenesis (Kaiser et al., 1989; Kloepper et al., 1991; Schippers et al., 1987). The strain A21-4 readily colonized on the pepper root system via seed and root inoculation and preoccupied possible infection sites.

S. plymuthica A21-4 inhibited successfully the pathogen's population in pepper root and rhizospere soil, which indicated that strain A21-4 was a strong rhizosphere competent biocontrol agent. The population density over than 10^6 cfu/g of soil is known sufficient to reduce the diseases (Shen et al., 2002). The strain A21-4 was well colonized in the rhizosphere of pepper plants and maintained enough population density to suppress the pathogens in the root and rhizosphere. It is presumed that additional treatment of cell suspension of A21-4 to rhizosphere after transplanting was needed to control the Phytophthora blight of pepper throughout growing season.

The potential antagonistic microorganisms selected by *in vitro* test often fail to effectively control plant diseases in greenhouse or field trials (Weller et al., 1985). Many antagonistic strains which showed strong antagonisms to *P. capsici in vitro*, sometimes were not effective in greenhouse or in field trials. But *S. plymuthica* A21-4 strongly inhibited the germination of cystospore, zoosprangia and mycelial growth of *P. capsici* not only in laboratory experiments, but also protected successfully the infection of pathogenic fungus in pot and small scale vinyl house experiments as well as commercial scale vinyl houses.

Acknowledgments

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References

Hur, J. M., Lee, Y. S., Kim, B. S. and Cho, C. H. 1990. Evaluation and Inheritance of Resistance to Phytophthora blight in Pepper. *Korean J. Plant Pathol.* 6:447-451.

Kim, Y. G. 1995. Biological Control of Phytpphthora Blight of Redpepper by Antagonistic *Bacillus Polymyxa* 'AC-1'. Dr. D. Thesis. Seoul National Uni. Korea.

Kaiser, W. J., Hannan, R. M. and Weller, K. M. 1989. Biological control of seed rot and pre-emergence damping-off of chickpea with fluorescent pseudomonads. *Soil. Biol. Biochem.* 21:269-273.

Kloepper, J. W., Zaboltowiz, R. M., Tipping, E. M. and Lifshitz, R. 1991. Plant growth promotion mediated by bacterial rhizosphere colonizers. In: The rhizosphere and plant growth. Edited by D. L. Keister and P. B. Cregan. pp. 315-326. Kluwer Academic Publishers, Cordrecht. the Netherlands.

Nam, C. G., Jee, H. J. and Kim, C. H. 1988. Studies on Biological Cotrol of Phytophthora Blight of Red-pepper . Enhancement of Antagonistic Activity by soil Amendment with Organic Materials. *Kor. J. Plant Pathol.* 4:313-318.

Park, K. S., Jang, S. W., Kim, C. H. and Lee, E. J. 1989. Studies on Biological Control of Phytophthora Blight of Red-pepper III. Formulations of *Trichoderma harzianum* and *Pseudomonas cepacia* Antagonistic to *Phytophthora capsici* and Their Preservation. *Kor. J. Plant Pathol.* 5:131-138.

Schippers, B., Bakker, A. W. and Bakker, P. A. H. M. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annu. Rev. Phytopathology* 25:339-358.

Schlub, R. L. 1983. Epidemiology of *Phytophthora capsici* on bell pepper. *J. Agric. Sci. Camb.* 100:7-11.

Shen, S. S., Kim, J. W. and Park, C. S. 2002. *Serratia plymuthica* strain A21-4: A Potential Biocontrol Agent Against Phytophthora Blight of Pepper. *Plant Pathol. J.* 18:138-141

Shen, S. S., Choi, O. H., Lee, S. M. and Park, C. S. 2002. *In vitro* and *In vivo* Activities of a Biocontrol Agent, *Serratia plymuthica* A21-4, Against *Phytophthora capsici*. *Plant Pathol. J.* 18:221-224

Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytophthol.* 26:379-407.