

In vitro* and *In vivo* Activities of a Biocontrol Agent, *Serratia plymuthica* A21-4, Against *Phytophthora capsici

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***In vitro* and *in vivo* activities of a biocontrol agent, *Serratia plymuthica* strain A21-4, was evaluated for the control of *Phytophthora* blight of pepper. Strain A21-4 inhibited mycelial growth, germination of zoosporangia and cystospores, and formation of zoospore and zoosporangia of *Phytophthora capsici* *in vitro*. In the pot experiment, incidence of *Phytophthora* blight of pepper in non-treated control was 100% at 14 days after inoculation, while no disease was observed in the plot treated with *S. plymuthica* A21-4. In the greenhouse test, infection rate of pepper in the non-treated plots was 74.5%, while it was only 12.6% in the plots treated with A21-4. Results indicate that *S. plymuthica* A21-4 is a potential biocontrol agent for *Phytophthora* blight of pepper.**

Keywords : biocontrol, *Phytophthora*, pepper, *Serratia plymuthica*.

Studies on the biological control of *Phytophthora* diseases in Korea are mostly focused on *Phytophthora* blight of pepper. Numerous isolates of actinomycetes, bacteria, and fungi that were found antagonistic to *P. capsici* have been selected in the past (Ahn and Hwang, 1992; Hwang and Kim, 1995; Jee et al., 1988; Kim and Hwang, 1992). Several studies have shown that promising antagonists markedly inhibited mycelial growth and zoosporangia germination of *P. capsici* on solid or in liquid media. Suspension of antagonistic microorganisms to naturally or artificially infested pot soil has also been tried to prove their biocontrol effects on *Phytophthora* blight of pepper.

A new promising biocontrol agent has been identified as *Serratia plymuthica* (Shen et al., 2002). The bacterium has been reported to be effective only in controlling *Phytophthora* and *Pythium* among several soilborne fungal pathogens tested. In this study, the biocontrol potentials of the bacterial isolate, A21-4, against *P. capsici* and the *Phytophthora* blight of pepper were evaluated *in vitro* and *in vivo*.

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Materials and Method

Antagonistic bacterium and the pathogen. *Serratia plymuthica* A21-4 was isolated from a rhizosphere soil of onion (*Allium cepa* L.). The strains of *S. plymuthica* were stored at -70°C in Tryptic Soy Broth (TSB) containing 20% glycerol. Strain A21-4 was grown at 28°C in TSB before use. The pepper blight pathogen, *Phytophthora capsici* Pa-61 (KACC 40476 NIAST), was grown on V8 juice agar (V8 juice 100 ml, distilled water 900 ml, CaCO₃ 1 g, agar 15 g). Five ml of sterile distilled water was added to the mycelial mat of *P. capsici* Pa-61 grown on V8 juice agar for 3 days and illuminated under a blue fluorescent light at 20-25°C for 16 h. In order to collect the zoosporangia, plates were chilled at 4°C for 10-30 minutes and returned to room temperature. Zoospores discharged by zoosporangia were harvested and adjusted to 10⁴ zoospore/ml.

Germination of zoosporangia and cystospores and formation of zoosporangia of *Phytophthora capsici*. A loopful bacterial mass of the strain A21-4 grown on TSA media for 48 h was suspended in 0.1 M MgSO₄ solution, and adjusted to 10⁸ cfu/ml. Inhibition of cystospore and zoosporangia germination of *P. capsici* Pa-61 by A21-4 *in vitro* was assayed on a slide glass. Cystospores and zoosporangial suspension of *P. capsici* (10⁴ spores/ml) was mixed with an equal volume of the bacterial suspension (10⁸ cfu/ml), and the mixture was dropped on a slide glass. The slide glasses were kept in a moistened petri plate at 30°C and incubated for 2 h. The number of germinated cystospores and zoosporangia were examined every 2 h.

The mycelial disc (8 mm in diameter) of *P. capsici* Pa-61 grown on V8 agar was transferred to a new empty petri plate, and 3 ml of the bacterial suspension (10⁸ cfu/ml) was dropped on the disc. Plates were incubated under a blue fluorescent light at 20-25°C for 16 h. The number of zoosporangia was directly counted under a microscope. The same amount of sterilized distilled water was used as control.

Suppression of *Phytophthora* blight of pepper. The roots of 50-day-old pepper seedlings (variety Nok-Kwang) obtained from commercial plug nursery were soaked in a suspension (10⁹ cfu/ml) of the strain A21-4 for 1 h and transplanted to pots (10 cm in diameter, 9 cm depth). One day after transplanting, 5 ml of zoospores suspension of *P. capsici* (10⁴ spores/ml) was inoculated into each pot. The inoculated plants were kept in a greenhouse bench and the number of infected plants was recorded.

The roots of 50-day-old pepper seedlings (variety Nok-Kwang) were soaked in the bacterial suspension (10⁹ cfu/ml) for 1 h and

Table 1. Effect of *Serratia plymuthica* A21-4 on the germination of cystospores and zoosporangia of *Phytophthora capsici*

Treatment	Germination rate (%) of <i>P. capsici</i>							
	cystospores				zoosporangia			
	2hr	4	6	8	2hr	4	6	8
A21-4	0	3.6	4.6	7.4	7.8	8.1	9.8	10.8
Water (control)	56.4	60.3	72.7	84.8	79.1	89.4	97.8	98.2

transplanted to a greenhouse. The plants were cultivated in the greenhouse using common farming practices.

Results

In vitro suppression of *Phytophthora capsici* by A21-4.

Strain A21-4 successfully inhibited germination of cystospores and zoosporangia of *P. capsici*. Germination rates of cystospores and zoosporangia were about 56% and 79% at 2 h after incubation in the sterilized DW, and almost 85% and 98% at 8 h after incubation, respectively. However, germination rates of cystospores and zoosporangia were only 7% and 11% at 8 h after incubation when treated with the bacterial strain A21-4 (Table 1 and Fig. 1).

The bacterial strain A21-4 successfully inhibited formation of zoosporangia and zoospore of *P. capsici*. When the mycelial disc of *P. capsici* was treated with the bacterial cell suspension, no zoosporangia were formed. Meanwhile, several zoosporangia were produced in the control treated with sterilized DW (Fig. 2).

Suppression of *Phytophthora* blight of pepper in pot.

Typical symptoms of *Phytophthora* blight began to appear

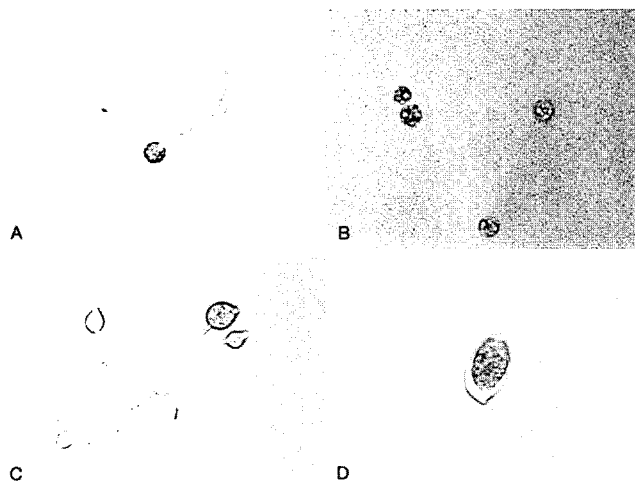


Fig. 1. Inhibition of growth and germination of cystospores and zoosporangia of *Phytophthora capsici* by *Serratia plymuthica* A21-4. A: Normal germination of cystospore; B: suppressed germination of cystospore by A21-4; C: Normal germination of zoosporangia; D: suppressed germination of zoosporangia by A21-4.

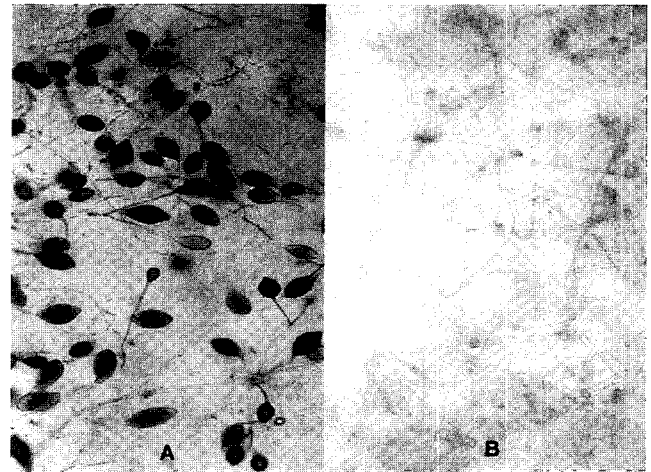


Fig. 2. Effect of *Serratia plymuthica* A21-4 on the formation of zoosporangia of *Phytophthora capsici* on V8 juice Agar. A: Untreated control; B: Treatment of A21-4 cell suspension (10^8 cfu/ml).

at 6 days after inoculation with *P. capsici*. Initial symptoms were drooping of one to all the leaves of the plant. At 14 days after inoculation, incidence of *Phytophthora* blight reached 100% in the untreated control with the strain A21-4. However, none of the plants were infected in the treated plots with strain A21-4 (Table 2 and Fig. 3).

Greenhouse experiment. The pepper seedlings treated with A21-4 and untreated control plants were transplanted in a greenhouse with soil naturally infested with *P. capsici*. One month after transplanting, infection rate of pepper blight was 12.6% in the treated plot with A21-4, and 74.5% in the non-treated control pepper plants. Results indicate that 83% of disease control efficiency was performed by *S. plymuthica* A21-4 (Figs. 4 and 5).

Table 2. Effect of *Serratia plymuthica* A21-4 on the disease incidence of *Phytophthora* blight of pepper in pots

Treatment	Disease incidence (%)				
	6 days	8	10	12	14
A21-4	0	0	0	0	0
non-treatment	33.3***	44.4**	66.7**	83.7**	100**

*The asterisk indicates significant difference by Duncan's multiple range test ($p=0.01$).



Fig. 3. Suppression of phytophthora blight of pepper by treatment of *S. plymuthica* A21-4. A: treatment of A21-4; B: Health control; C: Disease control.



Fig. 5. Over view of vinylhouse experiments. Healthy looking plants on the left are A21-4 treated and drooped and wilted plants on the right are untreated control.

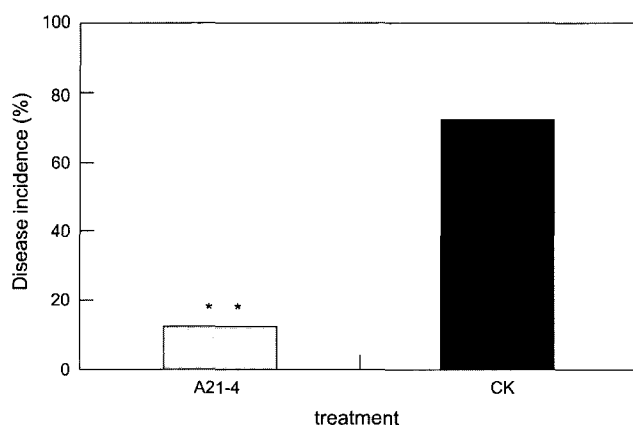


Fig. 4. Effects of *S. plymuthica* A21-4 on the disease incidence of Phytophthora blight of pepper in the vinylhouse. The asterisk indicates significant difference by Duncan's multiple range test ($p=0.01$). CK: untreated control.

Discussion

Some potential antagonistic microorganisms selected through *in vitro* tests often fail to effectively control plant diseases in the greenhouse or in field trials (Weller et al., 1985). However, *Serratia plymuthica* A21-4 not only strongly inhibited germination of cystospores, zoosporengia, and mycelial growth of *P. capsici* in the laboratory, but also successfully controlled infection of *P. capsici* to pepper in pots and in the greenhouse, showing 83% control efficacy.

Frankowski et al. (2001) reported that *S. plymuthica* produced a chitinase which inhibited the growth of several plant pathogens. They also suggested that chitinase production may be involved in the biological control mechanism of plant diseases. Most true fungi consist of chitin as the major structural component of cell wall (Frankowski et al.,

2001). However, pseudofungi such as *Phytophthora* and *Pythium* have cellulotic and β -glucans as the major structural component of cell wall (Ainsworth et al., 1973). In a previous study, *S. plymuthica* strain A21-4 did not inhibit the growth of *Fusarium* and *Rhizoctonia*, but strongly inhibited the growth of *Phytophthora* and *Pythium* (Shen et al., 2002). Therefore, results of this study suggest that chitinase may not be involved in the mechanism for the biocontrol activity of strain A21-4.

In conclusion, the results obtained in this study have shown that *S. plymuthica* strain A21-4 is a promising biocontrol agent for *Phytophthora* blight of pepper. However, more detailed and specialized studies, such as on the interaction with indigenous soil microorganisms and application method of the agent, are needed for effective and secure control of *Phytophthora* pepper blight in the fields.

Acknowledgment

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