Plant Pathol. J. 24(1) : 67-73 (2008)

The Plant Pathology Journal

Control of Crisphead Lettuce Damping-off and Bottom Rot by Seed Coating with Alginate and *Pseudomonas aeruginosa* LY-11

Kwang Ryool Heo[1], Kwang Youll Lee[1], Sang Hyun Lee[2], Soon Je Jung[3], Seon-Woo Lee[1] and Byung Ju Moon[1]*

[1]*Department of Applied Biology, College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea*
[2]*Department of Forest Environment, Korea Forest Research Institute, Seoul 130-712, Korea*
[3]*Department of Molecular Biotechnology, College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea*

(Received on January 15, 2008; Accepted on February 22, 2008)

Seedling damping-off and bottom rot caused by *Rhizoctonia solani* are yield limiting diseases of crisphead lettuce. To provide biocontrol measure in the management of the diseases, biocontrol strain *Pseudomonas aeruginosa* LY-11 was isolated from lettuce rhizosphere and introduced into crisphead lettuce rhizosphere by the seed coating delivery method. Alginate was used as a coating material to generate beads containing 10^6-$10^{6.5}$ colony-forming units (CFUs) of viable bacterial cells of LY-11. When seeds germinated from the alginate beads containing the strain LY-11, the bacteria established mostly in plant rhizosphere to maintain at least 10^4 CFU per gram of plant tissues. Crisphead lettuce seedlings germinated from the entrapped seeds were less affected from damping-off and bottom rot with disease control values of 70.4% and 85.4% respectively. Although *P. aeruginosa* LY-11 colonized plant rhizosphere and not phyllosphere, the result indicated that bottom rot caused by the foliar inoculation of *R. solani* was effectively reduced by the rhizobacteria. All data suggested that immobilized rhizobacterial application in seeds by alginate coating could control damping-off and induce induced systemic resistance of crisphead lettuce to reduce bottom rot.

Keywords : Alginate coating seed, Bottom rot, Crisphead lettuce, *Pseudomonas aeruginosa*, *Rhizoctonia solani*

Rhizoctonia solani Kühn (teleomorph; *Thanatephorus cucumeris* (Frank) Donk) is a common soilborne pathogen with a very wide host range including vegetables and many field crops. The pathogen primarily attacks below ground plant parts to cause damping-off, root rot and basal stem rot. However, the pathogen also infects above ground parts such as leaves to cause bottom rot and stem canker (Adam, 1988). *R. solani* infects crisphead lettuce (*Lactuca sativa* L., var. *capitata*) from the leaves to cause bottom rot and a report of bottom rot on lettuce was first reported from leaf-rotting disease of greenhouse lettuce in 1900 by Stone and Smith (1900). The disease has caused yield loss of up to 30% annually during early 1930s in organic soils and several anastomosis groups (AGs) of *R. solani* were associated with the bottom rot (Herr, 1992). Recently, the occurrence of crisphead lettuce bottom rot was also reported under greenhouse cultivation in Korea (Kim et al., 2004).

Disease management of crisphead lettuce bottom rot was mainly dependent on the fungicides. Although pencycuron developed to control *Rhizoctonia* diseases is still effective, pencycuron non-sensitive *R. solani* strains were also reported (Kim et al., 1996). Due to frequent development of fungicide resistance and increasing public concern about pesticide use, biological control has received much attention as an alternative way to control plant diseases (Cook, 1993). Biological control of soil-borne diseases uses natural antagonistic microorganisms with a number of beneficial traits (Haas and Keel 2003; Handelsman and Stabb, 1996). Some *Trichoderma* species and other fungal strains have been tested to control lettuce bottom rot disease caused by *R. solani* and showed the enhanced yield of plants (Coley-Smith et al., 1991; Grosch et al., 2006). One of the potential ways to control soilborne disease is to introduce the biocontrol agent on seeds. The application of biocontrol agent on crisphead lettuce seeds may provide the effective way to colonize and protect the plants (Russo et al., 1996). There are also reports on the development of induced systemic resistance (ISR) in various plants by the rhizosphere or seed introduced beneficial bacterium (Pieterse et al., 1996; Van Loon et al., 1998). Seed coating with antagonistic bacteria using suitable carrier material could allow bacteria to successfully colonize the crisphead lettuce plants and induce the systemic resistance against various plant diseases.

Alginate is a linear copolymer composed of 1,4-linked β-mannuronate and α-L-guluronate residues (Cheetham et al., 1979) and was considered as a biocompatible material to entrap microbial cells effectively and coat the plant seeds to deliver the agricultural inoculants (DeLucca et al., 1990; Fravel et al., 1985). Previous studies demonstrated that bacterial cells trapped by gelling materials such as alginate

*Corresponding author.
Phone) +82-51-200-7554, FAX) +82-51-200-7505
E-mail) bjmoon@dau.ac.kr

survived well and maintain the physiological activity (Davidson and Reuszer, 1978; Elsas et al., 1992; Paul et al., 1993; Trevors et al., 1993).

In this study, we isolated an antagonistic bacterium *Pseudomonas aeruginosa* LY-11 inhibiting mycelial growth of *R. solani* and delivered the bacterium on the plant rhizosphere using alginate seed coating. The delivered bacteria LY-11 showed effective control of crisphead lettuce damping off at seedlings stage and bottom rot in grownup plants. The effective prevention of bottom rot by the rhizosphere bacteria LY-11 suggested the ISR induction in crisphead lettuce against the foliar inoculation of pathogen.

Materials and Methods

Microorganisms, media and culture conditions. *P. aeruginosa* isolate LY-11 was routinely grown at 30°C on King's B medium (King et al., 1954) or in nutrient broth (NB) or nutrient agar (NA) if necessary. The plant pathogen *R. solani* PY-1 was previously isolated from bottom rot symptom of crisphead lettuce (Kim et al., 2004) was routinely grown at 25°C on potato dextrose agar (PDA) or potato dextrose broth (PDB) unless otherwise stated.

Isolation and identification of antagonistic bacteria. Bacterial strains were initially isolated from 3 different sites of lettuce fields of Myung-Gye Ri, Gyeongju, Korea. Plants were uprooted along with considerable amount of non-rhizosphere soil, brought immediately to the laboratory in polythene bags and air-dried. The non-rhizosphere soils were removed by gentle shaking, whereas the soil adhering strongly to the roots, referred as rhizosphere soil was collected. Ten grams soil from each sample was aseptically weighed and transferred to an 250 ml Erlenmeyer flask with 100 ml sterile water, and was shaken for 30 min at 150 rpm. Immediately after shaking, a series of 10-fold dilutions of the suspension was made for each sample by pipetting 1 ml aliquots into 9 ml sterile water. Diluted soil suspension were then dilute plated on King's B media to select fluorescent bacterial colonies. The fluorescent bacteria were selected 2 days after incubation at 28°C. Antifungal activities of fluorescent bacteria were tested on PDA against *R. solani* PY-1. Briefly, antagonistic bacteria were streaked at a distance of 5 cm from the agar discs (5 mm in diameter) of the target fungus *R. solani*. Fungal growth inhibition was determined 5-7 days after incubation at 25°C. Gram stain reaction of a selected strain LY-11 was performed by following the standard protocol and cell morphology was determined by light microscopy.

DNA sequencing of 16S ribosomal RNA gene. To amplify bacterial 16S ribosomal RNA (rRNA) gene, polymerase chain reaction (PCR) was performed using two universal primers, 8F (5'-AGAGTTTGATCCTGGCTCAG-3' *E. coli* position 8-27) and 1492R (5'-GGTTACCTTGTTACGACTT-3' *E. coli* position 1510-1492) (Edwards et al., 1989), and genomic DNA obtained from target isolate LY-11. The PCR amplification was carried out under the following conditions: 94°C for 3 min initial denaturation; 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min 30 sec, and final extension at 72°C for 5 min. The reaction mixture (final volume, 50 μl) contained 1 μl DNA (approximately 40-50 ng), 1X reaction buffer with 2 mM $MgCl_2$ and 2.5 unit of *Taq* DNA polymerase (Promega, Madison, USA). The amplified products were cloned into the pGEM-Teasy vector (Promega). The nucleotide sequencing was performed commercially at the DNA sequencing facility of GenoTech Corp. (Daejeon, Korea). DNA sequences of 16S rRNA gene were analyzed by using software from the Blastn program provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Immobilization of bacteria and seeds in alginates. *P. aeruginosa* LY-11 was cultured in King's B medium in a rotary shaker at 30°C for 24 to 48 hr to a final concentration of 10^9 colony forming unit (CFU)/ml. Entrapment of bacteria within beads was carried out under sterile conditions in a laminar flow hood with alginate as previously described by Russo et al. (1996). Bacterial suspension was prepared by washing stationary phase culture of LY-11 with 1/4 strength of diluent (NaCl, 8.5 g; KH_2PO_4, 0.3 g; Na_2HPO_4, 0.6 g; $MgSO_4$, 0.2 g; Gelatin, 0.1 g; distilled water, 1 liter). A 3% (w/v) solution of sodium alginate was prepared in distilled water and the solution was stirred overnight at room temperature. The bacterial culture was aseptically mixed with 3% (w/v) sodium alginate solution and stirred gently for 1 hr to give a final alginate concentration of 2.4%.

Crisphead lettuce seeds were purchased from Sakata Korea (Korea) and directly used for seed coating without further process. First, alginate solution with bacterial cells was sucked with 10 ml sterile syringe and a seed of crisphead lettuce was picked with the syringe containing the alginate solution. Second, the seed at the end of syringe was dropped into 0.1 M of calcium chloride solution by pushing alginate solution with syringe plunger. This process automatically encapsulated the seed with alginate solution to produce a bead containing a crisphead lettuce seed. The beads were maintained in the solution at room temperature for an additional 1 to 3 hr to obtain regular solid beads. The $CaCl_2$ solution was then pumped out, and the beads were washed twice with sterilized tap water. Control beads without antagonistic bacteria were prepared using 2.4% alginate solution mixed with 1/4 strength

diluent solution without bacteria.

Plant growth and fungal inoculation. Two greenhouse experiments were conducted to determine the effect of immobilized seeds with antagonistic bacteria in controlling damping off and bottom rot in crisphead lettuce. The coated crisphead lettuce (*Lactuca sativa* L.) seeds were sown in plug hole pots for damping-off experiment and in plastic pots (10 cm in diameter) for bottom rot experiment containing the commercial horticulture nursery media soil (Punong Co., Ltd, Korea). Pots were arranged in randomized complete blocks with five replications and two plants per pot. Disease incidence was recorded periodically and quantified as the percentage of crisphead lettuce plants rotted. The seeds were germinated in the soil and plants were maintained in a growth chamber at 22°C for 12 hr dark and 25°C for 12 hr light cycle until fungal inoculation.

Fungal inoculum of *R. solani* PY-1 was prepared in liquid culture in PDB. Briefly, 20 agar discs (5 mm in diameter) of actively growing fungus were inoculated in 200 ml PDB and incubated for 7 days at 25°C with 150 rpm. The fungal culture were then grounded in Warning blender and filtrated through cheese cloth to obtain fungal mycelium suspension. Final absorbance of the suspension was adjusted to 0.8 OD at 550 nm which can cause 50% disease rate when 1ml and 10 ml of the mycelium suspension per plant were challenged as foliar spray using a hand held garden sprayer at seedling stage and 6 weeks old plants respectively. Inoculated plants were maintained under 90% relative humidity at 24-25°C for 48 hrs and transferred to a growth chamber. Disease severity of damping-off was rated by counting the number of seedlings showing typical damping-off. Disease severity of bottom rot was assessed by estimating the percentage of diseased leaf area 4 days after pathogen inoculation. The disease control value was calculated using the following formula: Disease control value (%) = ((A–B)/A) × 100, where A is the disease severity triggered by pathogen inoculation alone and B is the disease severity after various treatments, including chemical fungicides or seed coating. For the bottom rot control treatments the fungicide Pencycuron® 20% (Dongbu Hannong Co., Korea) was used after 1,000-fold dilution with tap water.

Data from the disease control experiments were analyzed using analysis of variance (ANOVA) in a complete randomized design. Duncan's multiple range test was used to compare the means of the treatments in each experiment. All statistical analyses were conducted using SAS/STAT software (SAS Institute, 1989).

Plant colonization assay by *P. aeruginosa* LY-11. This experiment was performed in order to enumerate the number of CFU of *P. aeruginosa* LY-11 colonizing the plants of crisphead lettuce using the dilution plating technique. The alginate coated as well as uncoated seeds of crisphead lettuce were planted into non sterile commercial horticulture nursery media soil in pots and maintained with appropriate moisture to allow the seeds to germinate. The plants were maintained in growth chamber for 8 weeks at 22°C for 12 hr dark and 25°C for 12 hr light cycle. The grownup plants were uprooted carefully from the pot and loosely attached soils to plant root were removed by shaking smoothly. The three whole plants were segmented separately at every 15 mm from the tip of the plant to the end of root. This style of plant section divided leaves of plant into 4 parts and roots of plant into 6 parts with three replications. Fresh weight of each segment was measured and each segment was immediately suspended in sterile saline (0.8% NaCl) and vortexed for 5 min to allow bacterial cells to be released from plant tissues. Appropriate serial dilution of the bacterial suspensions was plated on NA to count the number of colonies.

Results and Discussion

Identification of an antagonistic bacterium *Pseudomonas aeruginosa*. We have plated soil suspension on various medium including King's B and selected several fluorescent bacteria. Since all of them were aerobic, they

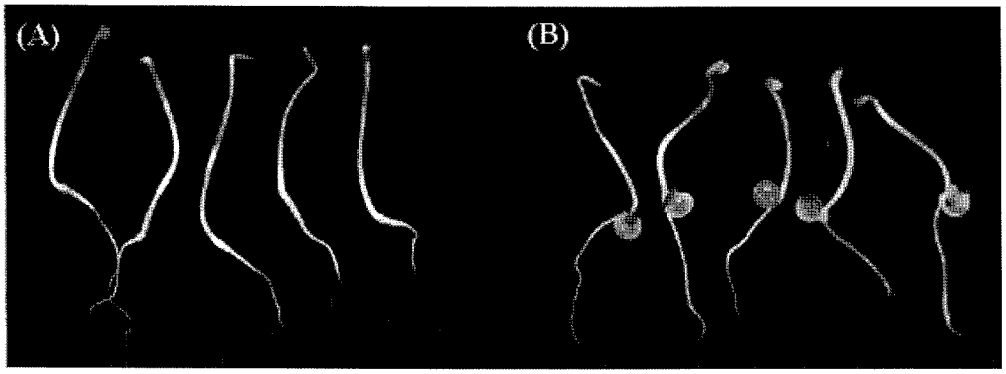

Fig. 1. Germination of crisphead lettuce seeds. (A), uncoated control; (B) coated seeds with alginate and *P. aeruginosa* LY-11.

were considered as fluorescent pseudomonads. All the selected fluorescent pseudomonads were subjected to fungal growth inhibition assay against *R. solani* PY-1. An isolate LY-11 showed the excellent fungal growth inhibition against *R. solani* PY-1 on PDA plate (data not shown). The fluorescent LY-11 isolate was typical Gram-negative aerobic, rod shape bacteria producing dark green pigment. Analysis of DNA sequences of 16S rRNA gene revealed that the isolate LY-11 was highly similar to *P. aeruginosa* with 99% DNA sequence identity (data not shown). Therefore, we identified the isolate LY-11 as *P. aeruginosa*, which produces the dark green pyocyanin pigments.

Bacterial coating on the crisphead lettuce seeds. Crisphead lettuce seed coating with alginate generated round beads containing seeds. The storage of coated beads at 4°C maintained the original shape over 7 days when sealed appropriately, while the beads were shrunken when stored at 4°C without sealing (data not shown). At higher temperatures, the seeds were germinated or beads become shrunken severely. The number of viable bacterial cells in the alginate beads, when stored at 4°C, was consistent up to 50 days (data not shown). Neither *P. aeruginosa* LY-11 nor alginate did affect crisphead lettuce seed germination and growth when compared to normal seed germination without bacterial application (Fig. 1), indicating that the coating material and biocontrol bacteria were not detrimental to plant growth and development. The number of bacterial cells in alginate beads with and without seeds was adjusted to 10^6-$10^{6.5}$ CFU of viable bacterial cells LY-11 during coating process. Further the alginate coating of seeds with *P. aeruginosa* LY-11 strain produced uniform beads containing one seed per bead, this immobilization process provided here is hand-made and has a limitation for automation.

Table 1. Population of *P. aeruginosa* LY-11 in the 4-week-old crisphead lettuce grown from alginate coated seeds

Section [a]		Log_{10} (CFU/g of tissue)[b]
Leaf	1	–
	2	5.99 ± 0.09 c
	3	7.83 ± 0.03 a
Root	4	7.11 ± 0.07 b
	5	6.05 ± 0.06 c
	6	6.01 ± 0.04 c

[a]Each section corresponds to 15 mm length plant segments from the top of plants. Section 1 and 4 correspond to the top of leaves and the top of roots, respectively.

[b]The numbers indicate the means from three replications and standard deviations. In a column, means followed by the same letter are not significantly different at the 5% level by DMRT.

Colonization of crisphead lettuce rhizosphere by *Pseudomonas aeruginosa*. Data revealed that the association of *P. aeruginosa* LY-11 was found to be more effective in crisphead lettuce plants (Table 1). Differences between inoculated treatments and the controls were visible in all experiments. In all assays, plants developed from alginate coated seeds performed more health than uncoated. Bacterial colonization of crisphead lettuce germinated from coated seeds were estimated by counting the number of viable LY-11 cells from randomly selected crisphead lettuce leaves and roots at 4 week- and 8 week-old plants. In the initial stage of plant growth, *P. aeruginosa* LY-11 colonized the above ground plant tissues (Table 1), but as the plant matures the bacterial cells were not detected from the above ground part, the growing leaves. However, the LY-11 was well established in plant rhizosphere to maintain more than 10^6 CFU per gram of soil (data not shown). At later stage of plant growth (8 week-old stage), bacteria mostly colonized plant rhizosphere (data not shown) along with the growing

Table 2. Population of *P. aeruginosa* LY-11 in the 8-week-old crisphead lettuce grown from alginate coated seeds

Section [a]		Log_{10} (CFU/g of tissue)[b]
Leaf	1	–
	2	–
	3	–
	4	6.50 ± 0.04 a
Root	5	5.26 ± 0.13 c
	6	5.40 ± 0.09 b
	7	5.08 ± 0.04 d
	8	4.32 ± 0.09 f
	9	4.26 ± 0.11 f
	10	4.49 ± 0.04 e

[a]Each section corresponds to 15 mm length plant segments from the top of plants. Section 1 and 5 correspond to the top of leaves and the top of roots, respectively.

[b]The numbers indicate the means from three replications and standard deviations. In a column, means followed by the same letter are not significantly different at the 5% level by DMRT.

Table 3. Disease control value of crisphead lettuce seeds coated with sodium alginate and *P. aeruginosa* LY-11 against damping-off caused by *R. solani* PY-1

Treatment	Disease control value (%)[a]
Coated seed + *R. solani* PY-1	70.4 (13.89) b
Uncoated seed + *R. solani* PY-1	0 (5.25) c
Uninoculated coated seed	100 (0.0) a
Uninoculated normal seed control	100 (0.0) a

[a]In a column, means followed by the same letter are not significantly different at the 5% level by DMRT. Numbers in parenthesis indicates the standard deviation from three replications.

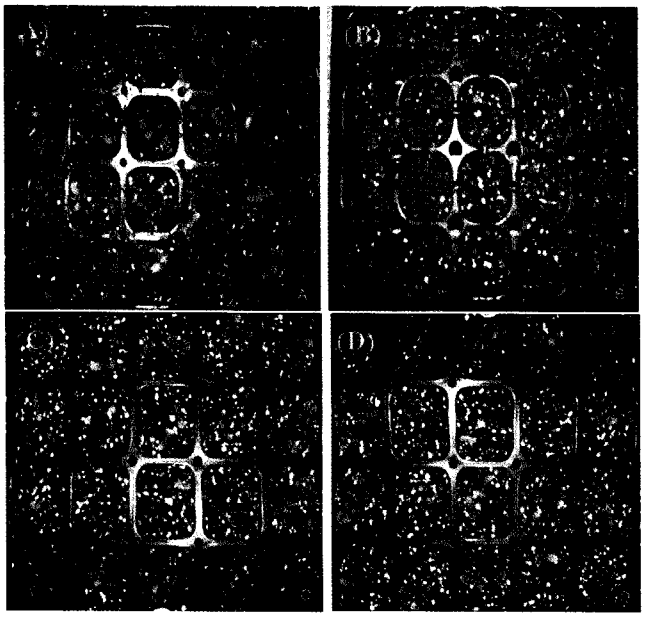

Fig. 2. Control of seedling damping-off of crisphead lettuce by seed coating with *P. aeruginosa* LY-11 at the plug pot experiments. The photo was taken 7 days after *R. solani* PY-1 inoculation. (A), Seedlings grown from coated seeds and fungus was inoculated; (B), Seedlings grown from uncoated seeds and fungus was inoculated; (C), Seedlings grown from coated seeds and un-inoculated control; (D), Seedlings grown from uncoated seeds and uninoculated control.

plant roots to maintain more than 10^4 CFU per gram of plant tissue (Table 2). In contrast, plant phyllosphere was almost free of LY-11 colonization.

Since *P. aeruginosa* LY-11 was initially isolated from lettuce grown fields, the rhizosphere colonization of crisphead lettuce by LY-11 reflects the bacterial adaptation in lettuce rhizosphere environments. The successful establishment of LY-11 in crisphead lettuce rhizosphere could play a role in plant protection from soil borne disease such as damping-off caused by *R. solani*.

Table 4. Disease control effect of coated seeds against bottom rot caused by *R. solani* PY-1 on crisphead lettuce in growth chamber

Treatment	Disease control value (%) 4 days after inoculation[b]
Coated seed + *R. solani* PY-1[a]	85.4 (7.22) b
Coated seed + Pencycuron + *R. solani* PY-1	98.9 (0.0) a
Coated seed	100.0 (0.0) a
Uncoated seed + *R. solani* PY-1	0.0 (0.0) c
Uncoated seed + Pencycuron + *R. solani* PY-1	97.5 (0.0) a
Uncoated seed	100.0 (0.0) a

[a]Pathogen inoculation, *R. solani* PY-1

[b]In a column, means followed by the same letter are not significantly different at the 5% level by DMRT. The number in parenthesis indicates the standard deviation from three replications.

Biological control of damping-off on crisphead lettuce. Disease control effect of seed coating of cripshead lettuce using alginate and rhizobacteria *P. aeruginosa* LY-11 was investigated by assessing the damping-off disease on seedlings of 3 days after germination. Disease control value in seedlings from alginate coated seeds was 70.4% (Table 3), while seedlings from uncoated seeds were completely infected 4 days after fungal inoculation (Fig. 2). Seedlings from coated with only alginate did show the similar damping-off to those from uncoated seeds (data not shown). Control experiments without fungal inoculation on both coated and uncoated seeds showed any damping off symptoms (Fig. 2C, 2D). Although heavy inoculation of pathogen caused seedling damping-off in seed coating treatment, seedlings from coated seeds with LY-11 strain exhibited good disease control effect. This result indicated that the LY-11 strain maintains its biocontrol activity in the germinated plants to suppress seedling damping-off.

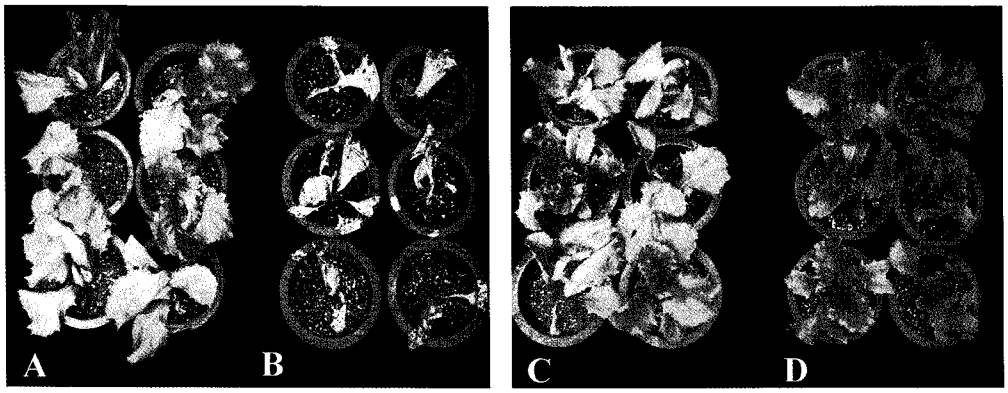

Fig. 3. Disease control effect of seed coating by alginate using *P. aeruginosa* LY-11 against bottom rot caused by *R. solani* PY-1 on crisphead lettuce in growth chamber. (A), Coated seeds with the pathogen treatment; (B), Uncoated seeds treated with the pathogen; (C), Coated seeds control; (D), Uncoated seeds control.

Biological control of bottom rot on crisphead lettuce. In another experiment, immobilized cells coated with seeds were also found to be effective to control bottom rot on crisphead lettuce. Disease control value from coating seeds was 85.4% (Table 4), while seedlings from uncoated seeds were completely destroyed 4 days after fungal inoculation (Table 4 and Fig. 3). The initial symptom development on the leaves was the same to the original symptoms of bottom rot observed on crisphead lettuce in the field (Kim et al., 2004). Plant leaves from coated with only alginate did show the similar disease lesion to those from uncoated seeds (data not shown). Control experiments without fungal inoculation on both coated seeds and uncoated seeds showed any bottom rot symptoms (Table 4). Chemical fungicide Pencycuron® treatment prior to pathogen inoculation control the bottom rot almost completely to show disease control value of 98.9% and 97.5% on plants from both coated seeds and uncoated seeds, respectively (Table 4).

Although disease control value (85.4%) on the plants from coated seeds was significantly different from that by pencycuron treatment, it was interesting that leaves of crisphead lettuce was not severely affected by fungal foliar spray inoculation on plants from coated seeds with *P. aeruginosa* LY-11. The disease control effect on the coated seeds could be explained by ISR induction from rhizosphere colonized LY-11. Since the LY-11 did not colonize crisphead lettuce phyllosphere, the direct antagonistic effect by *P. aeruginosa* LY-11 was not possible to suppress *R. solani* infection. Previously, several reports demonstrated that application and successful colonization of biocontrol strain in plant rhizosphere could prevent foliar disease occurrence (Duijff et al., 1998; Leeman et al., 1995; Pieterse et al., 1996; Van Peer et al., 1991; Van Wees et al., 1997). This type of disease reduction is through plant-mediated mechanism that is similar to pathogen induced systemic acquired resistance (SAR). The ISR mediated by rhizobacteria is very similar to SAR in terms of its systemic activation of plant disease resistance extending to aboveground plant parts (Van Loon et al., 1998; Pieterse et al., 2001).

P. aeruginosa LY-11 was introduced in crisphead lettuce rhizosphere by seed coating in this study and it successfully reduced foliar infection of *R. solani* resulting in reduced occurrence of bottom rot. This is probably due to ISR induction by the root colonized LY-11. Many ISR strains also promote plant growth (Bae et al., 2007; Kloepper and Schroth, 1981) and here we also observed the slightly enhanced plant growth from *P. aeruginosa* introduced plants (data not shown). To prove the ISR by LY-11, it is necessary to test with other foliar pathogen and to show the induction of genes involved in SAR. Taken together, this study showed that alginate based delivery of rhizobacteria was effective to control both soilborne disease and foliar disease. Despite of biocontrol effectiveness by *P. aeruginosa* LY-11 in seed coating, commercial development of seed coating technique by alginate is technically limited due to barrier to produce large amount of uniform beads containing one seed per bead. The process provided here is handmade and has a limitation for automation.

Acknowledgements. This study was supported by the Dong-A University Research Fund in 2007 and the Technology Development Program for Agriculture from the Agricultural R & D Promotion Center of the Ministry of Agriculture and Forestry of the Republic of Korea.

References

Adam, G. C. 1988. *Thanatephorus cucumeris* (*Rhizoctonia solani*) a species of wide host range. In: *Advances in Plant Pathology (Vol. 6). Genetics of Plant Pathogenic Fungi.* ed. by G. S. Sidhu, pp. P535-552. Academic Press, New York, USA

Bae, Y-S., Park, K., Lee, Y-G. and Choi, O-K. 2007. A simple and rapid method for functional analysis of plant growth promoting rhizobacteria using the development of cucumber adventitious root system. *Plant Pathol. J.* 23:223-225.

Cheetham, P. S. J. Blunt, K. W. and Bucke, C. 1979. Physical studies on cell immobilization using calcium alginate gels. *Biotechnol. Bioeng.* 21:2155-2168.

Coley-Smith, J. R., Ridout, C. J., Mitchell, C. M. and Lynch, J. M. 1991. Control of bottom rot disease of lettuce (*Rhizoctonia solani*) using preparations of *Trichoderma viride*, *T. hazianum* or toclofos-methyl. *Pl. Pathol.* 40:359-366.

Cook, R. J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. *Annu. Rev. Phytopathol.* 31:53-80.

Davidson, F. and Reuszer, H. W. 1978. Persistence of *Rhizobium japonicum* on the soybean seed coat under controlled temperature and humidity. *Appl. Environ. Microbiol.* 35:94-96.

DeLucca II, A. J., Connick Jr., W. J., Fravel, D. R., Lewis, J. A. and Bland, J. M. 1990. The use of bacterial alginates to prepare biocontrol formulations. *J. Ind. Microbiol.* 6:129-134.

Duijff, B. J., Pouhair, D., Olivain, C., Alabouvette, C. and Lemanceau, P. 1998. Implication of systemic induced resistance in the suppression of fusarium wilt of tomato by *Pseudomonas fluorescens* WCS417r and by nonpathogenic *Fusarium oxysporum* Fo47. *Eur. J. Plant Pathol.* 104:903-910.

Edwards, U., Rogall, T., Blocker, H., Emde, M. and Bottger, E. C. 1989. Isolation and direct complete determination of entire genes. *Nucleic Acids Res.* 17:7843-7853.

Elsas, J. D. van, Trevors, J. T., Jain, D. K., Wolters, A. C., Heijnen, C. E. and van Overbeek, L. S. 1992. Survival of, and root colonization by, alginate-encapsulated *Pseudomonas fluorescens* cells following introduction into soil. *Biol. Fertil. Soils* 14:14-22.

Fravel, D. R., Marois, J. J., Lumsden, R. D. and Connick Jr., W. J.

1985. Encapsulation of potential biocontrol agents in an alginate-clay matrix. *Phytopathology* 75:774-777.

Grosch, R., Scherwinski, K., Lottmann, J. and Berg, G. 2006. Fungal antagonists of the plant pathogen *Rhizoctonia solani*: selection, control efficacy and influence on the indigenous microbial community. *Mycological research*. 110:1464-1474.

Haas, D. and Keel, C. 2003. Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu. Rev. Phytopathol.* 41:117-153.

Handelsman, J. and Stabb, E. V. 1996. Biocontrol of soilborne plant pathogens. *Plant Cell* 8:1855-1869.

Herr, L. J. 1992. Characteristics of *Rhizoctonia* isolates associated with bottom rot of lettuce in organic soils in Ohio. *Phytopathology* 82:1046-1050.

Kim, H. T., Kamakura, T. and Yamaguchi, I. 1996. Effects of pencycuron on the osmotic stability of protoplasts of *Rhizoctonia solani*. *J. Pestic. Sci.* 21:159-163.

Kim, H-J., Park, J-Y., Back, J-W., Lee, J-W, Jung, S-J. and Moon, B. J. 2004. Occurrence of bottom rot of crisphead lettuce caused by *Rhizoctonia solani* and its pathogenicity. *J. Life Sci.* (In Korean) 14:689-695.

King, E. O., Ward, M. K. and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Clin. Med.* 44:301-307.

Kloepper, J. W. and Schroth, M. N. 1981. Relationship of in vitro antibiosis of plant-growth promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology* 71:1020-1024.

Leeman, M., Van Pelt, J. A., Den Ouden, F. M., Heinsbroek, M., Bakker, P. A. H. M. and Schippers, B. 1995. Induction of systemic resistance by *Pseudomonas fluorescens* in radish cultivars differing in susceptibility to fusarium wilt, using a novel bioassay. *Eur. J. Plant Pathol.* 101:655-664.

Paul, E., Fages, J., Blanc, P., Goma, G. and Pareilleux, A. 1993. Survival of alginate-entrapped cells of *Azospirillum lipoferum* during dehydration and storage in relation to water properties. *Appl. Microbiol. Biotechnol.* 40:34-39.

Pieterse, C. M. J., Van Pelt, J. A., Van Wees, S. C. M., Ton, J., Léon-Kloosteriziel, K. M., Keurentjes, J. J. B., Verhagen, B. W. M., Knoester, M., Van der Sluis, I., Bakker, P. A. H. M. and Van Loon, L. C. 2001. Rhizobacteria-mediated induced systemic resistance: triggering, signaling and expression. *Eur. J. Plant Pathol.* 107:51-61.

Pieterse, C. M. J., Van Wees, S. C. M., Hoffland, E., Van Pelt, J. A. and Van Loon, L. C. 1996. Systemic resistance in Arabidopsis induced by biocontrol bacteria is independent of salicylic acid and pathogenesis-related gene expression. *Plant Cell* 8:1225-1237.

Russo, A., Moënne-Loccoz, Y., Fedi, S., Higgins, P., Fenton, A., Dowling, D. N., O'Regan, M. and O'Gara, F. 1996. Improved delivery of biocontrol *Pseudomonas* and their antifungal metabolites using alginate polymers. *Appl. Microbiol. Biotechnol.* 44:740-745.

Stone, G. E. and Smith, R. E. 1900. The rotting of greenhouse lettuce. *Mass. Agric. Exp. Stn. Bull.* 69.

Trevors, J. T., Elsas, J. D. van, Lee, H. and Wolters A. C. 1993. Survival of alginate-encapsulated *Pseudomonas fluorescens* cells in soil. *Appl. Microbiol. Biotechnol.* 39:637-643.

Van Loon, L. C., Bakker, P. A. H. M. and Pieterse, C. M. J. 1998. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol.* 36:453-483.

Van Peer, R., Niemann, G. J. and Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. *Phytopathology* 81:728-734.

Van Wees, S. C. M., Pieterse, C. M. J., Trijssenaar, A., Van Westende, Y. A. M., Hartog, F. and Van Loon, L. C. 1997. Differential induction of systemic resistance in Arabidopsis by biocontrol bacteria. *Mol. Plant-Microbe Interact.* 6:716-724.