BioControl (2006) 51:339–351
DOI 10.1007/s10526-005-2934-x

Control of late blight (*Phytophthora capsici*) in pepper plant with a compost containing multitude of chitinase-producing bacteria

Dong Hyun CHAE[1], Rong De JIN[1], Hoon HWANGBO[1], Yong Woong KIM[1], Yong Cheol KIM[2], Ro Dong PARK[1], Hari B. KRISHNAN[3] and Kil Yong KIM[1,*]

[1]*Division of Applied Bioscience and Biotechnology, and Environmental-Friendly Agriculture Research Center, Chonnam National University, Gwangju, 500-757, Republic of Korea;* [2]*Division of Applied Plant Science, College of Agriculture and Life Science, Chonnam National University, Gwangju, 500-757, Republic of Korea;* [3]*Plant Genetics Research Unit, USDA-ARS, University of Missouri, Columbia, MO, USA*

**Author for correspondence: e-mail: kimkil@chonnam.ac.kr*

Received 11 July 2004; accepted in revised form 8 September 2005

Abstract. Compost sustaining a multitude of chitinase-producing bacteria was evaluated in a greenhouse study as a soil amendment for the control of late blight (*Phytophthora capsici* L.) in pepper (*Capsicum annuum* L.). Microbial population and exogenous enzyme activity were measured in the rhizosphere and correlated to the growth and health of pepper plant. Rice straw was composted with and without a chitin source, after having been inoculated with an aliquot of coastal area soil containing a known titer of chitinase-producing bacteria. *P. capsici* inoculated plants cultivated in chitin compost-amended soil exhibited significantly higher root and shoot weights and lower root mortality than plants grown in pathogen-inoculated control compost. Chitinase and β-1,3-glucanase activities in rhizosphere of plants grown in chitin compost-amended soil were twice that seen in soil amended with control compost. Colony forming units of chitinase-producing bacteria isolated from rhizosphere of plants grown in chitin compost-amended soil were 10^3 times as prevalent as bacteria in control compost. These results indicate that increasing the population of chitinase-producing bacteria and soil enzyme activities in rhizosphere by compost amendment could alleviate pathogenic effects of *P. capsici*.

Key words: β-1,3-glucanase, chitinase-producing bacteria, pepper, *Phytophthora capsici*

Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable crop in Korea. Continuous mono-cropping has led to an increased incidence of disease such as late blight caused by *Phytophthora capsici*. This pathogenic fungus occurs worldwide, causing root and

crown rot, aerial blight of leaves, stems, and fruits, resulting in marked reduction in the yield of pepper plants (Mao et al., 1998). To date, synthetic chemical fungicides have been the most efficacious method used to control this disease. Use of these fungicides has been associated with deterioration of soil physico-chemical properties, accumulation of toxic compounds, and an increase in pathogen resistance (Hwang and Kim, 1995). Considering the above problems associated with the use of chemical fungicides, a search for a more effective and environmentally favorable strategy to minimize deleterious effects of *P. capsici* was deemed prudent. Biological control, the use of microorganisms to prevent plant diseases, offers an attractive alternative without the negative impact of chemical control. Therefore, biological control tactics have become an important approach to facilitating sustainable agriculture (Wang et al., 2002). Recently researchers have recognized that cultivating plants in composts provides a degree of control of soil-borne pathogens. Several mechanisms have been proposed to explain the suppression of plant diseases by composts. Beneficial chemical components in compost (Cronin et al., 1996), stimulation of microbial communities antagonistic to pathogens (Brito Alvarez et al., 1995), and induction of systemic acquired resistance have been implicated in the suppression of plant disease (Zhang et al., 1998). Pathogen suppressive properties of a compost depends on several factors, such as the type of biomass composted, the composting process, stability of the products, application time and compost dose (De Ceuster and Hoitink, 1999).

Chitin, a natural polymer of N-acetyl-D-glucosamine (Glc-Nac) residues linked by β-1-4 bonds, is exceeded in abundance only by the biopolymer cellulose. Chitin and its derivatives hold great economic value because of their versatile biological activities and agrochemical applications (Hirano, 1996; Wang et al., 1999). Addition of chitin to the soil may be expected to stimulate soil microbial activities through the promotion of species capable of degrading the polymer. This has been confirmed by the observed increase in chitinase and glucanase activity in chitin-treated soils and increase in population of specific soil-borne microorganisms (Muzzarelli, 1977; Mian et al., 1982).

The objectives of this research were to evaluate the population of chitinase-producing bacteria and exogenous enzyme activities in soil amended with a compost harboring multitude of chitinase-producing bacteria, and to ascertain whether this protocol was efficacious in minimizing or negating the deleterious effect *P. capsici* on pepper plants.

Material and methods

Preparation of the chitin compost and control compost

Chitin compost is composed of 30% crab shell, 20% vermiculite, 40% rice straw, and 10% rice bran. This compost was mixed with coastal area soil (compost: soil = 99.9:0.1, w/w) containing chitinase-producing bacteria (10^6 g^{-1} soil). Control compost comprised of 20% vermiculite, 40% rice straw, and 40% rice bran was also combined with the coastal soil in the same ratio. Composts were kept in a covered 100-l jar for one year. Moisture content was maintained between 45 and 60% and composting mixture was aerated at 0.7 MPa for 30 min once per week.

Antifugal activity of chitin compost

Five kilogram of chitin compost or control compost were suspended in 5 l of tap water individually and stirred thoroughly. These mixtures were allowed to filtrate through a single layer of cheesecloth, and passed through Whatman No. 6 filter paper. The filtered extract (chitin or control compost water extract) was mixed with distilled water in 1:9 ratio (v/v) to prepare PDA (Potato Dextrose agar) medium. After autoclaving, one plug of nonsporulating *P. capsici* mycelium which was grown at 27 °C on PDA medium for 5 days, was cut with a stainless borer and inoculated into the PDA medium containing chitin or control compost water extract. Inhibited growth of *P. capsici* caused by chitin compost water extract or control compost water extract in PDA medium was investigated at 5 days after incubation at 27 °C.

Plant growth conditions and plant harvest

Soil was mixed with vermiculite and sand (2:1:1, v/v/v) for tray and pot experiments. Chemical characteristics of the mixture are presented in Table 1. The mixture was placed in 20-cell trays (125 cm^3Cell^{-1}). Pepper seeds (Chungok, Nongwoo Seed Co.) were surface disinfected for 1 min with 70% ethanol, rinsed five times with sterile distilled water and planted one per cell. Four weeks after planting, pepper seedlings were transplanted to pots containing 700 g of the soil mixture amended with 30% chitin compost or control compost. Chemical properties of both chitin and control composts are shown in Table 2. Plants were grown in a greenhouse at 25–30 °C and 60–70% relative

Table 1. Chemical properties of the culture medium mixed soil, vermiculite and sand in 2:1:1 ratio

pH (1:5 H_2O)	EC (dS m^{-1})	T-N (%)	O.M. (%)	Av P_2O_5 (mg kg^{-1})	CEC ($cmol^+$ kg^{-1})	Exc. cations ($cmol^+$ kg^{-1})		
						Ca	K	Mg
6.31	0.2	0.3	0.9	31.85	8.94	3.12	0.34	1.85

humidity. Tissue samples were harvested at 0, 1, 3, 5, 7, and 9 days after pathogen inoculation (see below), washed in running tap water, rinsed in deionized water, and weighed.

Inoculation

P. capsici (KACC 40483), obtained from the Korea Agricultural Culture Collection (Suwon, Korea), was cultured without light in V8 agar for 3 days at 30 °C. Cultures were then flooded with sterile deionized water and incubated under continuous fluorescent light for 3 days at 25 °C to induce sporangia production. Zoospores were released by chilling flooded cultures to 4 °C for 1 h, followed by incubation at 25 °C for 30 min. Mycelia and sporangial debris were removed from zoospore suspension by filtration through four layers of sterile cheesecloth (Kim and Hwang, 1994). Zoospore populations were quantified using a rose Bengal agar (Wollum, 1982). Zoospore suspensions were diluted to 1×10^6 ml^{-1} with sterile deionized water prior to inoculation of pepper plants. Eight weeks after planting, 10 ml of zoospore suspension was poured into each of five 1 cm×4 cm deep holes located equidistant from the growing pepper plants.

Preparation of colloidal chitin

Colloidal chitin used in chitin agar plates and in the determination of chitinase activity was prepared by the modified method of Godoy

Table 2. Chemical properties of the culture medium after mixing with 30% of chitin or control compost

Treatment	T-N (%)	O.M. (%)	C/N	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})
Ccom	1.02	25.33	14.40	3.9	24.9	18.7
Tcom	1.01	24.55	14.10	3.95	24.7	18.4

Ccom: chitin compost; Tcom: control compost

et al. (1982). Fifty grams of finely ground (0.25 mm) crustacean chitin (DA 2.63%) was dissolved in 600 ml 85% (w/w) phosphoric acid at 25 °C. The solution was passed through a 0.5 mm mesh sieve, and 2 l of tap water was added to form a colloidal suspension, which was allowed to settle overnight. Excess supernatant fluid was decanted and the chitin suspension was dialyzed against running tap water for 96 h and then in distilled water for 24 h. The resulting chitin suspension had a pH of 6.0. Percentage of chitin in the suspension was gravimetrically determined by drying a 50 ml aliquot at −70 °C for 24 h. The suspension was maintained in darkness at 4 °C until use.

Root mortality assay

Root mortality was measured using the modified method of Liu and Huang (2000). Approximately 250 mg of fresh roots were incubated with 5 ml 0.6% 2,3,5-triphenyltetrazolium chloride in 50 mM phosphate buffer (pH 7.4) for 24 h in darkness at 30 °C. Formazan from water rinsed roots was twice extracted with 95% ethanol at 70 °C for 4 h. Extracts were combined and adjusted to a final volume of 20 ml with 95% ethanol. Absorbance of the solution was read spectrophotometrically at 490 nm. A standard curve relating formazan content to mass of actively metabolizing tissue was constructed by assaying samples comprised of different proportions of living and autoclaved root tissue. Root mortality was expressed as the percentage of dead root dry weight to the total root weight.

Soil enzyme activity

β-1,3-glucanase (EC 3.2.1.6) activity was determined using the modified method of Tabatabai (1982), which measures glucose produced as the result of laminarin digestion (Yedidia et al., 2000). One gram of soil from rhizosphere was mixed with 0.25 ml of toluene, 4 ml of 50 mM NaOAc buffer (pH 5.0) and 1 ml of 0.5% laminarin in a test tube and kept at 37 °C for 2 h. After this period, 1 ml 0.5 M of $CaCl_2$ and 4 ml of 0.5 M NaOH was added to the solution, and mixed thoroughly. The mixture was centrifuged at 3000 rpm for 20 min and filtered through Whatman No. 2 paper to collect the soil-free supernatant. A 1.5-ml aliquot of dinitrosalicylic acid was added to 500µl of filtered mixture, and then the reaction was stopped by heating in boiling water for min. β-1,3-glucanase activity was immediately measured at 550 nm using a spectrophotometer. The activity was calculated from a standard curve obtained from glucose. One

unit of activity was defined as the amount of enzyme that liberated 1μmol of glucose per hour at 37 °C.

Chitinase (EC 3.2.1.14) activity was determined using the modified method of Tabatabai (1982), which measures the amount of N-acetyl glucosamine produced from colloidal chitin (Yedidia et al., 2000). One gram of soil collected from the rhizosphere was mixed with 0.25 ml of toluene, 4 ml of 50 mM NaOAc buffer (pH 5.0) and 1 ml of 0.5% colloidal chitin in a test tube and kept in 37 °C for 2 h. After this period, 1 ml 0.5 M of $CaCl_2$ and 4 ml of 0.5 M NaOH was added and mixed thoroughly. The mixture was centrifuged at 3000 rpm for 20 min and filtered through a Whatman No. 2 paper to obtain the soil-free supernatant. A 1.0 ml aliquot of Schales' reagent was added to 0.75 ml of filtered mixture, and then the reaction was stopped by heating in boiling water for 15 min. Chitinase activity was immediately measured at 420 nm using a spectrophotometer. The activity was calculated from a standard curve obtained from N-acetyl glucosamine. One unit of activity was defined as the amount of enzyme that liberated 1μmol of N-acetyl glucosamine per hour at 37 °C.

Microbial populations

Microbial population of the rhizosphere was enumerated by the soil dilution plate method (Wollum, 1982). Soil samples were incubated in a basal medium containing 1% colloidal chitin as a sole carbon. The compositions of the basal medium were 0.2% Na_2HPO_4, 0.05% KNO_3, 0.1% KH_2PO_4, 0.1% NH_4Cl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% $CaCl_2 \cdot 2H_2O$, 0.01% yeast extract, 1% colloidal chitin, and agar 2%. After 7 days incubation at 30 °C, chitinase-producing bacteria developed clear zones around colonies. After seven days incubation at 30 °C, pellucid zones appeared around colonies of chitinase-producing bacteria.

Results

Antifugal activity of chitin compost and control compost

Suppression of *P. capsici* on plate caused by chitin compost water extract and control compost water extract is shown in Figure 1. The PDA medium containing chitin compost water extraction greatly inhibited growth of *P. capsici* compared to control compost water extract.

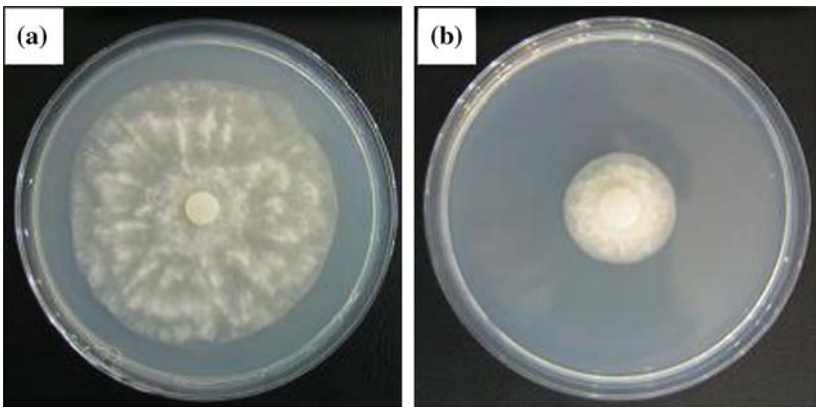

Figure 1. Growth of *P. capsici* on PDA medium containing 10% control compost (a) and chitin compost water extract (b) at 27 °C for 5 days.

Plant fresh weight and root mortality

Shoot and root fresh weights of plants growing in soil amended with either control or chitin compost were greater than that of plants cultivated in the respective *P. capsici* inoculated soil. Plant shoot and root weights were 36% and 54% greater in the *P. capsici* inoculated chitin compost soil than in control compost soil which harbored the organism (Figure 2). Late blight symptoms were significantly suppressed in plants growing in soil amended with chitin compost. Root mortality was determined to be 87% less in plants growing in the inoculated chitin soil than in those from the inoculated control (Figure 3). Furthermore, pepper plant disease severity was markedly reduced in chitin-amended soil (Figure 4). Nine days after the inoculation, plants grown in the inoculated control soil exhibited leaf chlorosis and wilt and root necrosis. However, plants in pathogen-inoculated chitin soil appeared normal (Figure 4).

Microbial activities in rhizosphere

Rhizosphere of pepper plants from both inoculated and non-inoculated chitin soil exhibited two-fold higher chitinase activity than was observed in control plants grown under the same conditions. Nine days after introduction of *P. capsici*, rhizosphere chitinase activity in inoculated chitin amendment was 65% more than that in inoculated control (Figure 5a). Activity of β-1,3-glucanase in rhizosphere of pepper grown in inoculated and non-inoculated chitin soil was 1.5 times greater than

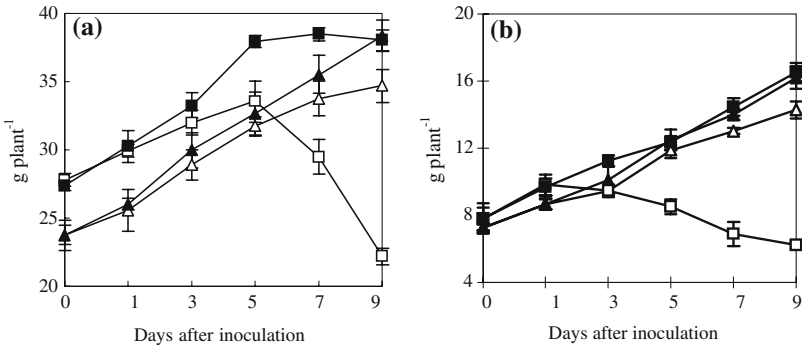

Figure 2. Change in shoot (a) and root (b) fresh weight in pepper plants as influenced by Chitin compost (Ccom) (-▲-), Chitin compost + *P. capsici* (Ccom + Phy) (-△-), Control compost (Tcom) (-■-), and Control compost + *P. capsici* (Tcom + Phy) (-□-). Mean value of 3 replicates. Bars represent standard error.

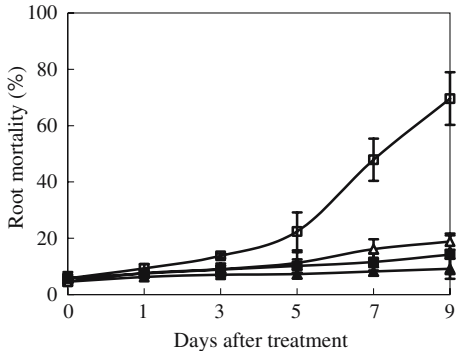

Figure 3. Change in root mortality in pepper roots as influenced by Chitin compost (Ccom) (-▲-), Chitin compost + *P. capsici* (Ccom + Phy) (-△-), Control compost (Tcom) (-■-), and Control compost + *P. capsici* (Tcom + Phy) (-□-). Mean value of 3 replicates. Bars represent standard error.

that of inoculated and non-inoculated control (Figure 5b). The colony forming units of chitinase-producing bacteria in rhizosphere of plants grown in either inoculated or non-inoculated chitin soils treatments was 10^3 times that seen in the control compost soils (Figure 5c).

Discussion

We evaluated the effect of chitin compost on both plant growth and soil enzyme activity and its ability to suppress disease symptoms

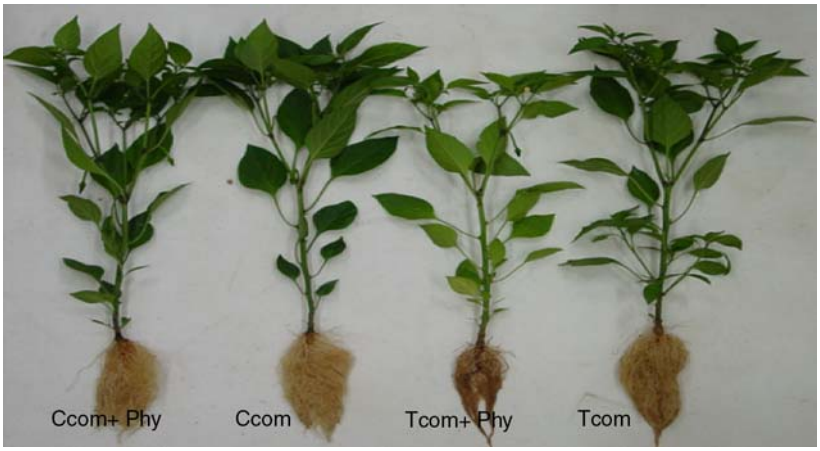

Figure 4. Pepper plants growth as influenced by Chitin compost (Ccom), Chitin compost + *P. capsici* (Ccom + Phy), Control compost (Tcom), and Control compost + *P. capsici* (Tcom + Phy).

caused by *P. capsici*. Plants grown in the pathogen-inoculated control compost-amended soil exhibited the lowest fresh weight of shoots and roots with significant root mortality. Symptoms of *P. capsici* infection were visible within nine days of exposure to the pathogen. However, pathogen-subjected plants growing in the chitin compost-amended soil did not exhibit symptoms of *P. capsici* induced late blight.

As reported elsewhere, disease control in amended soil may be the cumulative result of complex interactions between host-plant and pathogen organisms. Relationships among pathogen, the soil environment, organic amendments and plant health are obviously complex and make it difficult to assess the activities occurring in soil. However, the most important factor in the control of a soil-borne disease is to adapt the antagonistic microorganisms to the soil environment and to keep the superior position. Mian et al. (1982) reported that an addition of chitin to soil stimulated the growth of bacterial species with chitinolytic properties. Furthermore, inhibition of fungal growth was more effective when β-1,3-glucanase and chitinase acted synergistically (Lim et al., 1991; Lorito et al., 1993). In the current study, both soil enzyme activities (chitinase and β-1,3-glucanase) revealed quite higher values with the chitin compost amendment compared to the control compost amendment. As a result, we can suggest that the increased microbial activities in rhizosphere with chitin compost amendment may act as a main factor to protect pepper plants from *P. capsici* infection.

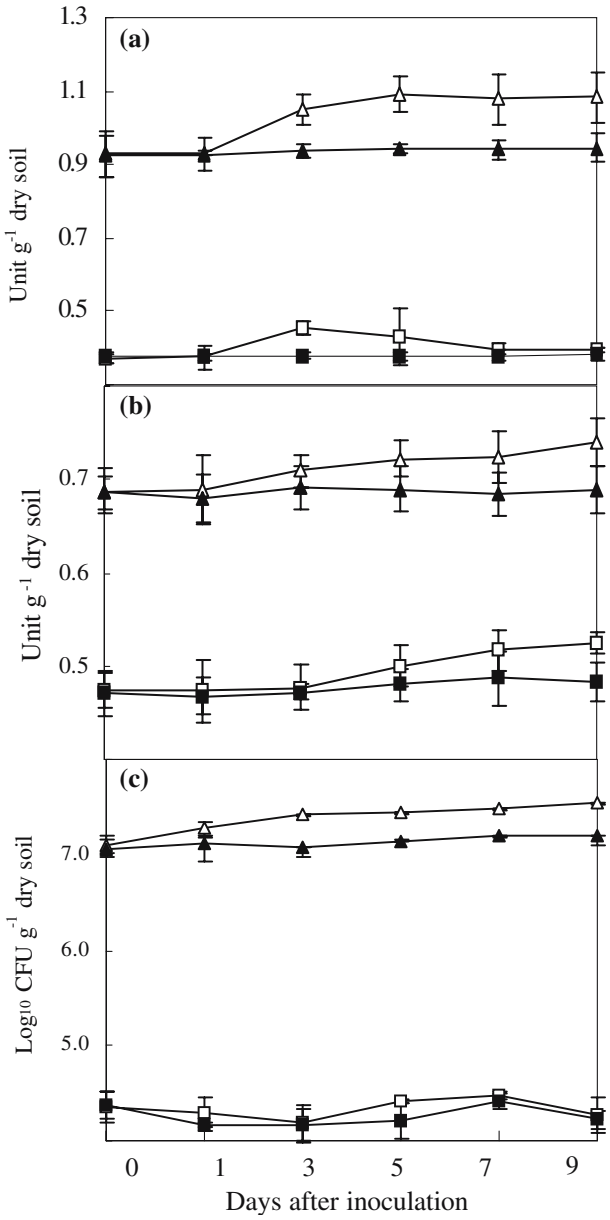

Figure 5. Change in chitinase activity (a), β-1,3-glucanase activity (b) and chitinse-producing bacteria (c) in soil as influenced by Chitin compost (Ccom) (-▲-), Chitin compost + *P. capsici* (Ccom + Phy) (-△-), Control compost (Tcom) (-■-), and Control compost + *P. capsici* (Tcom + Phy) (-□-). Mean value of 3 replicates. Bars represent standard error.

Except soil enzymes, non-protein materials such as antibiotic compounds are also highly effective in reducing *P. capsici* infection according to our investigation (Figure 1). Antibiotic compounds produced by microorganisms in rhizosphere of plants growing in soil amended with chitin compost have been shown to have beneficial effects on the protection of plants from diseases. Several reports have indicated that particular Gram-positive bacteria exhibit potential in the biological control of oomycetes through production of antibiotics (Toussait et al., 1997; Emmert and Handelsman, 1999). Compost amended with shrimp shell containing chitin polymer promoted the proliferation of Gram-positive bacteria, a group of bacteria producing more than 70% of known antibiotics (Strohl, 1997). In the chitin compost the principal bacteria found are *Bacillus* sp, *Penibacillus* sp., and *Serratia* sp. (data not shown). Secretion of chitinase and antibiotics by *Bacillus* sp. facilitates utilization of chitin as a carbon source by the bacterium (Wang et al., 2002). Guelder et al. (1988) reported that the iturins produced by *B. subtilis* acted as an antifungal substance against peach brown rot disease. Akihiro et al. (1993) showed that *B. subtilis* NB22 and *B. subtilis* RB14 produced antifungal peptides, iturin A and surfactin. Studies in our laboratory have shown that *B. subtilis* HJ927 released 3-methylbutyric acid, 2-methylbutyric acid, and methyl 2-hydroxy, 3-phenylpropanoate, which strongly inhibited *P. capsici* (Lee, 2003).

In conclusion, chitin compost-amended soil increased soil enzyme activities and population of chitinase-producing bacteria in rhizosphere of pepper plants. The technique was efficacious in minimizing *P. capsici* pathogenicity. Therefore, chitin compost could provide an alternative to synthetic chemicals for pathogen control in specific agricultural crops.

Acknowledgements

This study was supported by a grant (Code#20050401034716) from BioGreen 21 program, Rural Development Administration and Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

References

Akihiro, O., A. Takashi and S. Makoto, 1993. Prodution of the antifungal peptide antibiotic, iturin by *Bacillus subtilis* NB22 in solid state fermentation. *J. Ferment. Bioeng*. 75: 23–27.

Cronin, M.J., D.S. Yohalem, R.F. Harris and J.H. Andrews, 1996. Putative mechanisms and dynamics of inhibition of the apple scab pathogen *Venturia inaequalis* by compost extracts. *Soil Biol. Biochem.* 28: 1241–1249.

De Brito Alvarez, M.A., S. Gagne and H. Antoun, 1995. Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. *Appl. Environ. Microbiol.* 61: 194–199.

De Ceuster, T.J.J. and H.A.J. Hoitink, 1999. Prospects for composts and biocontrol agents as substitutes for methyl bromide in biological control plant disease. *Compost Sci. Utili.* 7: 6–15.

Godoy, G., R. Rodriguez-Kabana and G. Morgan-Jones, 1982. Parasitism of eggs of *Heterodera glycines* and *Melodogyne arenaria* by fungi isolated from cysts of *H. glycines*. *Nematropica* 19: 111–119.

Emmert, E.A.B. and J. Handelsman, 1999. Biocontrol of plant disease: a (Gram-) positive perspective. *FEMS Microbiol. Let.* 171: 1–9.

Gueldner, R.C., C.C. Reilly, P.L. Pusey, C.E. Costello, R.F. Arrendale, R.H. Cox, D.S. Himmelsbach, F.G. Crumley and H.G. Cutler, 1988. Isolation and identification of iturins as antifungal peptides in biological control of peach brown rot with *Bacillus subtilis*. *J. Agri. Food. Chem.* 36: 366–370.

Hirano, S., 1996. Chitin biotechnology application. *Biotech. Ann. Review* 2: 237–258.

Hwang, B.K. and D.H. Kim, 1995. *Phytophthora* blight of pepper and its control in Korea. *Plant Dis.* 79: 221–227.

Kim, Y.J. and B.K. Hwang, 1994. Differential accumulation of β-1,3-glucanase and chitinase isoforms in pepper stems infected by compatible and incompatible isolates of *Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 45: 195–209.

Lee, H.J., 2003. Biocontrol of late blight in pepper (*Capsicum annuum* L.) by antagonistic *Bacillus subtilis* HJ927. A Master's Thesis, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, Republic of Korea.

Lim, H.S., Y.S. Kim and S.D. Kim, 1991. *Pseudomonas stutzeri* YPL-1 genetic transformation and antifungal mechanism against *Fusarium solani*, an agent of plant root rot. *Appl. Environ. Microbiol.* 57: 510–516.

Liu, X.Z. and B.R. Huang, 2000. Carbohydrate accumulation in relation to heat stress tolerance in two creeping bentgrass cultivars. *J. Am. Soc. Hortic. Sci.* 125: 442–447.

Lorito, M., A.D. Pietro, C.K. Hayes, S.L. Woo and G.E. Harman, 1993. Antifungal, synergistic interaction between chitinlytic enzymes from *Trichoderma harzianum* and *Enterobacter cloacae*. *Mol. Plant Pathol.* 83: 721–728.

Mao, W., J.A. Lewis, R.D. Lumsden and K.P. Hebbar, 1998. Biocontrol of selected soilborne disease of tomato and pepper plants. *Crop Prod.* 17: 535–542.

Mian, I.H., G. Godoy, R.A. Shelby, R. Rodriguez-Kabana and J.G. Morgan, 1982. Chitin amendments for control of *Meloidogyne arenaria* in infested soil. *Nematropica* 12: 71–84.

Muzzarelli, R.A., 1977. *Chitin*. Pergamon Press, New York 309.

Strohl, W.R., 1997. Industrial antibiotics: today and the future. In: W.R. Strohl, (ed), *Biotechnology of Antibiotics*. Marcel Dekker Ins., New York. pp. 3–35.

Tabatabai, M.A., 1982. Soil Enzymes. In: A.L. Page, R.H. Miler and D.R. Keeney (eds), *Method of soil analysis. Part 2. Chemical and Microbiological Properties*. American Society of Agronomy Madison, Wisconsin, USA. pp. 903–947.

Toussaint, V., D. Valois, M. Dodier, E. Faucher, C. Dery, R. Brzezinski, L. Ruest and C. Beaulieu, 1997. Characterization actinomycetes antagonistc to *Phytophthora fragariae* var. *rubi*. *Phytoprotection* 78: 43–51.

Wang, S.L., T.C. Yieh and I.L. Shih, 1999. Production of antifungal compounds by *Pseudomonas aeruginosa* K-187 using shrimp and crab shell powder as a carbon source. *Enzy. Micro. Technol.* 25: 142–148.

Wang, S.L., I.L. Shih, C.H. Wang, K.C. Tseng, W.T. Chang, Y.K. Twu, J.J. Ro and C.L. Wang, 2002. Production of antifungal compounds from chitin by Bacillus subtilis. *Enzy. Micro. Tech.* 31: 321–328.

Wollum, A.G., 1982. Cultural methods for soil microorganism. In: A.L. Page, R.H. Miler and D.R. Keeney (eds), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. American Society of Agronomy Madison, Wisconsin, USA. pp. 781–02.

Yedidia, I., N. Benhamou, Y. Kapulnik and I. Chet, 2000. Induction and accumulation of PR protein activities during early stages of root colonization by the mycoparasite *Trichoderma harzuanum* strain T-203. *Plant Physiol. Biochem.* 38: 863–873.

Zhang, W., D.Y. Han, W.A. Dick, K.R. Davis and H.A.J. Hoitink, 1998. Compost and compost water extract-induced systematic acquired resistance in cucumber and *arabidopsis*. *Phytopathology* 88: 450–455.