

## Biocontrol of pepper diseases by *Lysobacter enzymogenes* LE429 and Neem Oil

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A chitinolytic bacterium having a strong antagonistic activity against various pathogens including *Phytophthora capsici* was isolated from rhizosphere soil, and identified as *Lysobacter enzymogenes* (named as LE429) based on 16S rRNA gene sequence analysis. This strain produced a number of substances such as chitinase,  $\beta$ -1, 3-glucanase, lipase, protease, gelatinase and an antibiotic compound. This antibiotic compound was purified by diaion HP-20, sephadex LH-20 column chromatography and HPLC. The purified compound was identified as phenylacetic acid by gas chromatography-electron ionization (GC-EI) and gas chromatography-chemical ionization (GC-CI) mass spectrometry. In field experiment, pepper plants were treated by the strain LE429 culture (CB), neem oil solution (NO), combination (CB+NO) or control (CON). Plant height and number of branches, flowers and pods of pepper plant in CB treatment were generally highest, and followed by CB+NO, CON and NO. The fungal pathogens were strongly inhibited, while several insect pests were discovered in CB treatment. Any insect pests were not found, while all fungal pathogens tested were not suppressed in NO treatment. However, in CB+NO treatment, non incidence of fungal pathogens and insect pests were found. The strain LE429 producing secondary metabolites with neem oil should be a potential agent to control fungal diseases and insect pests.

**Key words:** *Lysobacter enzymogenes* LE429, Lytic enzyme, Phenylacetic acid, Chitinase, Antifungal activity, Neem oil

### Introduction

Pepper (*Capsicum annuum* L.) is a popular and widely grown crop in the world. Production of pepper plant is seriously hampered by severe attacks of various insects and fungi. A wide variety of insect pests can cause severe damages to pepper plants, which are beetles, caterpillars, aphids, pepper weevils and thrips. Weevil can be a serious pest of pepper fruit and thrips can damage pepper leaves, fruit and flowers by feeding them (Shipp et al., 1998). Aphids are known to serve as vectors for several serious

virus diseases in pepper plants. In addition, damping-off disease caused by fungal pathogen *Pythium* spp. in pepper crops is economically very important worldwide (Whipps and Lumsden, 1991). Rapid germination of sporangia of *Pythium* after exposure to exudates or volatiles from seeds makes management of the pathogen very difficult. Late blight caused by *Phytophthora capsici* leading to rots in both stem and root of pepper plant (Ueeda et al., 2006), and *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. vasinfectum (Ma, 2001) are soil-borne fungal diseases, widely distributed throughout the world, and markedly reduce the pepper yield.

Management of these diseases and insect pests of pepper has been carried out using chemicals and resistant cultivars. However, use of resistant cultivars is very expensive, and

chemicals involve risks to the environment, which may be lethal to useful soil insects and beneficial microorganisms in the rhizosphere, and enter the food chain (Kim and Chsung, 2004). Therefore, it is necessary to develop alternative ways of plant disease control. One alternative is a biological control which uses microorganisms having ability to antagonize pathogens as well as a botanical insecticide neem.

Recently, a great deal of attention has been devoted to search biocontrol approaches using microorganisms due to high specificity against the targeted plant pathogens, easy handling of mass production and low cost (Shoda, 2000). Kim and Chung (2004) reported that an antagonistic microorganism inhibited soil borne disease, *colletotrichum lagenarium*. Cho et al. (2009) found that larva mortality of Diamondback Moth, *Plutella xylostella* (L) increased, while the number of laid eggs decreased by *Bacillus thuringiensis*.

Members of the genus *Lysobacter* are typically found in soil, and characterized by gliding motility and ability to degrade other microorganisms such as fungi and bacteria as well as nematodes. This genus also produced both antibiotics and enzymes which are well-established traits in biocontrol mechanisms (Handelsman and Stabb, 1996). *Lysobacter enzymogenes* can produce extracellular enzymes such as chitinases and  $\beta$ -1,3-glucanases that are capable of degrading the major cell wall components of pathogenic fungi (Sullivan et al., 2003). Folman et al. (2003) reported that *L. enzymogenes* 3.1T8 produced lytic enzymes such as protease and lipase which exhibited antibiosis against *Phythium aphanidermatum* and other phytopathogens. Strain *Lysobacter* sp. SB-K88, the root-colonizer of spinach and tomato, produced xanthobaccins A, B and C which inhibited *Aphanomyces cochlioides*, a pathogen of damping-off (Islam et al., 2005). These evidences suggest that the members of this genus are potential biocontrol agents for soil borne plant pathogens and pests. Neem (*Azadirachta indica*, A. Juss. (L)) of the family of the Meliaceae has been investigated intensively for its potential as bio-insecticide (El Shafie and Basedow, 2003). Oil extracts of neem seeds, and water and ethanol extracts of neem leaves are known to inhibit the growth of a great variety of insects (Amadioha, 2000). Use of neem extract with more than 300 known components affects the physiology and behaviour of a wide range of insects, mites and nematodes (Schaaf et al., 2000). It is widely used around the world today either as in an alone treatment (Kumar et al., 2005) or in combination with synthetic pesticides or entomopathogens

(Depieri et al., 2005).

The objectives of this work were *in vitro* to isolate the antagonistic bacterial strain against fungal pathogens, to investigate the production of antibiotics and lytic enzymes from *L. enzymogenes* LE429, and to investigate control ability against fungal diseases and insect pests by the strain LE429 and neem oil at field in Myanmar.

## Materials and Methods

**Selection and identification of antagonistic bacterial strain** Soils were collected from a field enriched with crab shell in Yeonggwang, Korea. The soils were serially diluted with sterile water until a rate of  $10^{-6}$ , and then the mixtures were inoculated on chitin agar (CA) medium containing 0.5% colloidal chitin, 0.2%  $\text{Na}_2\text{HPO}_4$ , 0.1%  $\text{KH}_2\text{PO}_4$ , 0.05%  $\text{NaCl}$ , 0.1%  $\text{NH}_4\text{Cl}$ , 0.05%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.05%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.05% yeast extract and 2% agar, and then incubated at 30 °C for 5 days. Colonies having clear zones (5 mm in diameter) were selected, and successively examined for antifungal activity against *P. capsici* on chitin-potato dextrose (CPD) agar medium (0.25% colloidal chitin, 0.1%  $\text{Na}_2\text{HPO}_4$ , 0.05%  $\text{KH}_2\text{PO}_4$ , 0.025%  $\text{NaCl}$ , 0.05%  $\text{NH}_4\text{Cl}$ , 0.025%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.025%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.025% yeast extract, 0.5% potato dextrose broth and 2% agar). One strain having strong antifungal activity was selected for further study, and kept in 25% glycerol solution at -70 °C. The strain was identified by an analysis of the 16S rRNA gene sequence using blast search at gene bank data base of NCBI (Bethesda, MD).

**Production of lytic enzyme** To investigate activities of chitinase and  $\beta$ -1,3-glucanase, the bacterial culture supernatant was collected daily, and assayed by method of Lingappa et al. (1962), and Yedia et al. (2000), respectively. Also, protease activity was examined by observing the production of a halo zone surrounding colonies on skim milk agar (MA) medium, and lipase activity was detected by formation of colony on luria-bertani (LB) agar medium supplemented with 1% Tween 80, respectively (Folman et al., 2003). Gelatinase activity was confirmed by observing a clear zone with 30% trichloroacetic acid on gelatin agar medium (Patricia and Larry, 2007)

**Identification of antifungal substance** Bacterial strain was cultured at 30 °C for 3 days in tryptic soy broth (TSB), and then centrifuged at 6,000 rpm for 15 min. The super-

nant was adjusted to pH 3.0 with 1N HCl, and mixed with ethyl acetate (EtOAc). The EtOAc extract was concentrated using a vacuum rotary evaporator (Büchi, Switzerland), and then purified by Diaion HP-20 chromatography (Mitsubishi Chemical Co., Japan) with a stepwise gradient of water:methanol (100:0, 50:50, 30:70, 0:100, v/v). Eluted fractions of 70% methanol and 100% methanol, which had strong antifungal activities, were further purified by Sephadex LH-20 column chromatography (25-100 mesh, Sigma, Sweden) using 100% methanol as a running phase. The active fraction was more purified by HPLC with a C<sub>18</sub> reversed-phase column (Symmetry Prep C<sub>18</sub> 7.8 x 300 mm, Waters). The HPLC was performed with 70% acetonitrile at a flow rate of 1.2 ml min<sup>-1</sup> using a Shimadzu system. Each peak was detected at 210 nm by a SPD-10A UV-VIS detector (Shimadzu). The active compound collected by HPLC was confirmed using a gas chromatography-mass spectrometry (GC-MS, GCMS-Qp2010, Shimadzu, Japan) (Ko, 2009).

**Antagonism assay** The isolate was tested for antagonism toward several fungal pathogens such as *Phytophthora capsici*, *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani* using dual plate culture method in Myanmar (Crawford et al., 1993). A fungal disc of five days old mycelial mat of 0.5 cm diameter was placed in the centre of the petri plate containing CPD agar medium, and the isolate was streaked at a distance of approximately 3 cm from the fungal disc. The plate was incubated at 28°C for 5 days. Antagonism was determined as % antifungal activity, equal to  $[(A - B)/A \times 100]$ , where A is the mycelia growth of control fungi, and B is the mycelia growth of the fungi with bacterial inoculation. Antifungal activity, reported as % inhibition, is as follows: (-) below 10%, (±) 10–20%, (+) 20–50%, (++) 50–80% and (+++) 80–100%.

**Preparation of bacterial culture and neem oil** One L of the strain LE429 culture ( $8 \times 10^9$  CFU ml<sup>-1</sup>) and 1 kg of crab shell powder were added to a tank containing 1000 L of tap water, and incubated with air supply of 10 L min<sup>-1</sup> through air bubble machine at 30–35°C for 5 days. After the incubation of 5 days, 3 kg of complex fertilizer (N: P: K = 15: 15: 15, Kemira, Yara Thailand Co., Ltd) and 3 kg of sugar were added to the tank, and then incubated for 3 more days. Neem oil was extracted from the seeds of neem trees, *Azadirachta indica* A. Juss (Meliaceae) in Myanmar. The 110 ml of neem oil was thoroughly dissolved in 4.5 L

of water containing 45 g of detergent powder (Elan, Myanmar), which was called as neem oil solution.

**Field trial of pepper** All experiments were done at the field of Kyaukse Township, Mandalay Division, Myanmar on October 2009 to February 2010. Seeds of pepper were sown in nursery tray filled with commercial grade bedding soil. At 4 weeks after sowing, pepper seedlings were transplanted to a field. The experiments were conducted with 3 replications, using randomized block design. The size of plot was 3 x 3 m per plot where 30 pepper plants were grown. At 2, 4, 6, 10 and 12 weeks after transplanting, 2.5 L of diluted bacterial culture (CB, bacterial culture: tap water = 1 : 1) and the neem oil solution (NO) was sprayed to each plot. In CB+NO, 1.25 L of diluted bacterial culture and 1.25 L of neem oil solution were sprayed separately. Tap water was used as a control (CON). At 13, 14 and 15 weeks after transplanting, incidence of fungal diseases and insect pests was investigated, and also potential yield components were recorded. Flying insects were captured with a fine-meshed net, identified, and counted on each plant, while non-flying insects were hand collected, identified, and counted on each plant (Ortega, 1987).

**Statistical analysis** The data were separated by using Tukey's Studentized Range (HSD) test with an analysis of variance (ANOVA) assigned at  $P \leq 0.05$  by program of the Statistical Analysis System 9.1 (SAS, 2002).

## Results

**Identification of antagonistic bacterium and antifungal activity** A new strain was isolated from the field enriched with crab shells in Yeong gwang, Korea, and identified as *Lysobacter enzymogenes* (named LE429) based on the 16S rRNA sequence analysis (NCBI Accession No. EU668316). This strain showed the strong antifungal activity against *P. capsici* on chitin-patato dextrose (CPD) agar medium (Fig. 1A). Antifungal activity by *L. enzymogenes* LE429 against various fungal pathogens was performed with dual plate assay against several fungal diseases on CPD agar medium. *L. enzymogenes* LE429 exhibited 80–100% of strong antifungal activity against *P. capsici* as well as other fungal pathogens as listed in Table 1.

**Measurement of lytic enzyme activities** Protease activity on MA medium and gelatinase activity on a plate con-

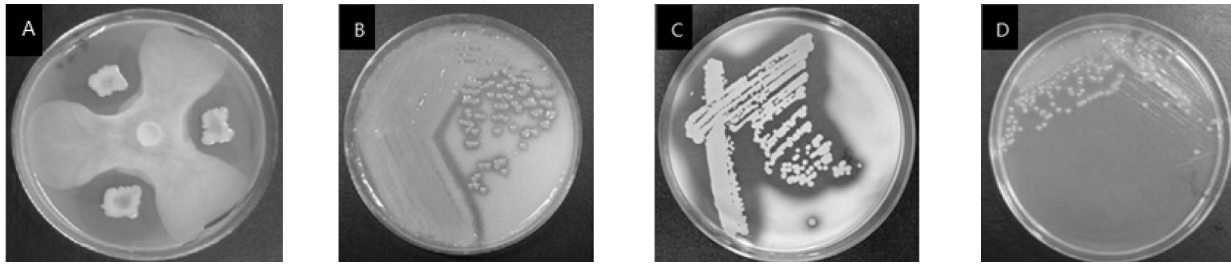


Fig. 1. Growth inhibition (A) of *P. capsici* induced by *Lysobacter enzymogenes* LE429 on CPD agar medium at 25°C for 7 days. Visible enzyme activities of the strain LE429 on each selective agar medium. The bacterium was grown on skim milk agar medium (protease, B), gelatin agar medium (gelatinase, C) and lipid agar medium with 1% Tween 80 (lipase, D) at 30°C for 3 days incubation.

Table 1. *In vitro* antifungal activity by *L. enzymogenes* LE429 against various fungal pathogens on CPD agar medium at 30°C for 7 days.

Fungi	Antifungal activity <sup>†</sup>
<i>Fusarium oxysporum</i>	+++
<i>Pythium aphanidermatum</i>	+++
<i>Rhizoctonia solani</i>	+++
<i>Phytophthora capsici</i>	+++

<sup>†</sup>Antifungal activity was reported as (±) between 10 and 20%, (+) between 20 and 50%, (++) between 50 and 80%, (+++) between 80 and 100%. CPD= colloidal chitin + potato dextrose broth.

taining 1% gelatin were detected by clear zone formation, respectively (Fig. 1B and C). Lipase activity was explained by the colony formation on LB agar medium supplemented with 1% Tween 80 (Fig. 1D). Production of lytic enzymes from the strain LE429 was examined during incubation periods. Chitinase activity markedly increased until 3 days with maximum value of 0.58 unit ml<sup>-1</sup>, and thereafter slowly decreased as shown in Fig. 2A. β-1,3-glucanase activity increased until 5 days with maximum value of 0.51 unit ml<sup>-1</sup>, and rapidly decreased (Fig. 2B).

**Characterization of antifungal substance** One of the purified compounds by HPLC indicated a single peak at retention time ( $t_R$ ) of 15.4 min, and showed strong antifungal activity against *P. capsici* when only 3 mg of pure compound was applied by disc diffusion method (results not shown). This compound was identified as phenylacetic acid (PAA) by GC-EI (Fig. 3A) and GC-CI (Fig. 3B).

**The incidence of insect pests and fungal diseases in pepper plantation in field** As shown in Table 2, fungal diseases such as *Fusarium* wilt and *Phytophthora* root & stem rot were discovered in 50-80%, while any fungal

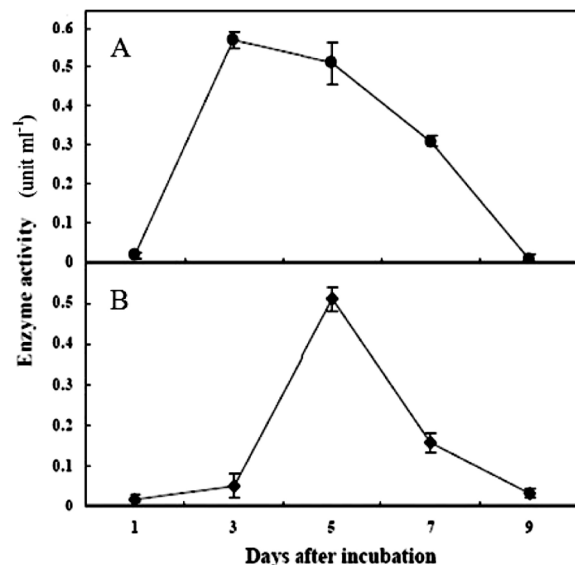


Fig. 2. Changes in chitinase (-●-, A) and β-1,3-glucanase activity (-◆-, B) produced by *Lysobacter enzymogenes* LE429 in the culture medium at 30°C for 9 days.

disease was not found in CB and CB+NO. The incidence result of insect pests in pepper was in Table 3. In NO and CB+NO treatment, incidence of insect pests was not found, but all insect pests were found in CON in which incidence of weevil was 5-10%, while incidence of aphid & thrips, borer and worm (army & fruit) was 10-20%. In CB, incidence of aphid & thrips and weevil was found in 5-10%, while incidence of borer and worm (army & fruit) was not found.

**Potential yield components of pepper in field** As shown in Table 4, the highest number of flowers and number of pods were found in CB treatment. The lowest value was generally found in NO treatment except number of branches. The plant height in CB, CB+NO and CON was higher than in NO. The number of branches was not different among all treatments.

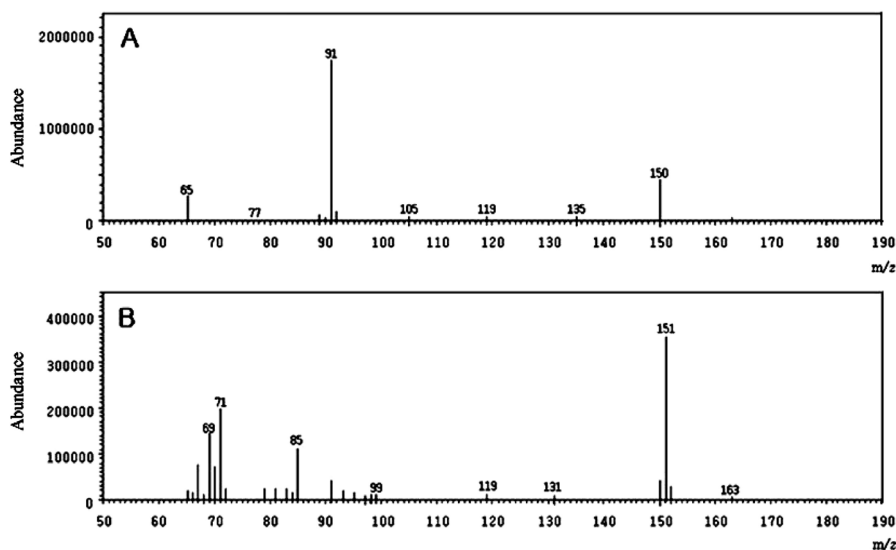


Fig. 3. GC-EI (A) and GC-CI (B) spectrum of phenylacetic acid (PAA) isolated from the culture filtrate of *L. enzymogenes* LE429 ( $t_R$  26.5 min of GC).

Table 2. The incidence of fungal diseases in pepper plantation during the observation of each treatment.

Treatment <sup>†</sup>	Fusarium wilt	Phytophthora root & stem rot	Pythium fruit rot
CB	-	-	-
NO	++	++	±
CB+NO	-	-	-
CON	++	++	±

<sup>†</sup>CB : bacterial culture, NO : neem oil solution, CB+NO : bacterial culture + neem oil solution, CON : water.

The incidence of fungal diseases was reported as (±) between 10 and 20%, (+) between 20 and 50%, (++) between 50 and 80%, (+++) between 80 and 100%.

Table 3. The incidence of insect pests in pepper plantation during the observation of each treatment.

Treatment <sup>†</sup>	Aphid & Thrips	Borer	Worm (army & fruit)	Pepper weevil
CB	±	-	-	±
NO	-	-	-	-
CB+NO	-	-	-	-
CON	+	+	+	±

<sup>†</sup>CB : bacterial culture, NO : neem oil solution, CB+NO : bacterial culture + neem oil solution, CON : water.

The incidence of insect pests was reported as (±) between 10 and 20%, (+) between 20 and 50%, (++) between 50 and 80%, (+++) between 80 and 100%.

Table 4. Result of potential yield components per pepper plant during the observation of each treatment.

Treatment <sup>†</sup>	Plant height (cm)	No. of branches (plant <sup>-1</sup> )	No. of flowers (plant <sup>-1</sup> )	No. of pods (plant <sup>-1</sup> )
CB	56.1a	11.7a	1942a	1655a
NO	38.6b	11.2a	1037d	892c
CB+NO	51.4a	11.2a	1390b	970b
CON	47.5ab	10.6a	1192c	967b

<sup>†</sup>CB : bacterial culture, NO : neem oil solution, CB+NO : bacterial culture + neem oil solution, CON : water.

Different letters indicate significant difference at  $P \leq 0.05$  of Tukey's HSD multiple comparison test.

## Discussion

There have been several reports on antifungal and insecticidal activity of antagonistic microorganisms, and use of them for plant disease control (Nakayama et al., 1999; Folman et al., 2003; Mohan and Gujar, 2001). Several microorganisms antagonistic to fungal pathogens and pests are known to exist in soils. A novel bacterium having strong antifungal activity against *P. capsici* (Fig. 1) was isolated from soil, and identified as *Lysobacter enzymogenes* LE429 based on the 16S rRNA sequence analysis (NCBI Accession No. EU668316).

*Lysobacter* sp. is known to produce various antibiotics such as xanthobaccins, tripropeptin, lysobactin and cephabacin (O'Sullivan et al., 1988; Hashizume et al., 2004). Xanthobaccins produced by *Stenotrophomonas* sp. strain SB-K88 played a key role in suppression of sugar beet damping-off disease (Nakayama et al., 1999). In our study, the antifungal substance produced by the strain LE429 in culture medium was identified as phenylacetic acid (PAA) (Fig. 3). The suppressive effect of crude extract containing PAA was pronounced in the *in vitro* test against *P. capsici* (results not shown). PAA is widely known as a natural auxin which is found in the shoots of plants (Pemadasa, 1982). In our previous studies, PAA was also found to have antibacterial and antifungal activity against various pathogens (Mao et al., 2006).

Indeed, many of the microbial enzymes including chitinase,  $\beta$ -1, 3-glucanase, lipase and protease can degrade fungal cell wall because fungal wall is a complex structure typically composed of chitin,  $\beta$ -1, 3 and  $\beta$ -1,6-glucan and proteins. Several studies have shown that antagonistic microorganisms excrete lytic enzymes that are responsible for their antifungal activities (Elad, 1982).

As shown in Fig. 1 and 2, we examined that strain LE429 produced protease, gelatinase, lipase, chitinase and  $\beta$ -1,3-glucanase, which may be closely associated with destruction of *Phytophthora capsici*, *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani* cell wall composed of glucans and other polymers with chitin (Table 1). Therefore, the lytic enzymes and antibiotic produced by the strain LE429 synergistically play an important role in the destruction of fungal pathogens. This finding is consistent with previous results (Woo et al., 2002) that the synergistic interaction between lytic enzymes and antifungal compounds produced by *Pseudomonas* spp. was involved in the antagonistic mechanism against several

pathogenic fungi. Schirmbock et al. (1994) reported that synergism of hydrolytic enzymes and peptaibol antibiotics may play an important role in the antagonistic properties of *Trichoderma harzianum* against fungal pathogens. In addition, our strain LE429 also produced indole acetic acid (IAA) in the culture broth during the experiment periods (results not shown). IAA produced by bacteria plays a major role in the development of host plant system which stimulates the growth of a primary root of canola seedling (Patten and Glick, 2002). Results from Dey et al. (2004) showed that IAA produced by *P. fluorescens* contributes to enhance plant growth as well as plant disease suppression.

Field trial studies provided an evidence for biocontrol activity which was confirmed with the comparison of *L. enzymogenes* LE429 and control. Evidence of fungal diseases in peppers was not found at bacterial culture (CB) and bacterial culture plus neem oil solution (CB+NO), while all insect pests were not discovered in NO and CB+NO (Table 2 and 3). When peppers inoculated with zoospores suspension of *P. capsici* were treated with a suspension of *Serratia plymuthica* A21-4, the number of diseased plants and disease severity were significantly reduced (Shen et al., 2002). Perez et al. (1986) reported that *Trichogramma* spp. was responsible for biological control agent against rice insect pests. In addition, insect growth and two aphid species population in ornamental plants were reduced by neem extracts (Koul, 1999). Among all treatments, the highest control ability against insect pests and fungal diseases was found in CB+NO. These results indicated that the combined treatment of the strain LE429 culture plus neem oil solution enhanced ability to control fungal diseases and insect pests synergistically. Mao et al. (1998) reported that a complex of corn diseases caused by several pathogenic fungi was reduced by the activity of an antagonistic fungus and bacterium working together. The combination of entomopathogenic nematodes with neem extracts could be of success in controlling *P. xylostella* on chinese cabbage plants in the field (Abdel-Razek and Gowen, 2002).

At the ripening stages, the most effective yield component in the peppers was found in CB, followed by CB+NO (Table 4). In CB+NO, plant height and number of flowers and pods were higher than those in NO. Mao et al. (1997) reported that fruit yield increased finally because damping-off of tomato and pepper caused by *Rhizoctonia solani* and *Pythium ultimum* could be biologically controlled by

### *Gliocladium virens* and *Burkholderia cepacia*.

In conclusion, *L. enzymogenes* LE429 produced phenylacetic acid and lytic enzymes such as  $\beta$ -1,3-glucanase, chitinase, protease, gelatinase and lipase. According to the *in vitro* and field trial result, the strain LE429 can control several fungal diseases and insect pests, and also promote the plant growth. Neem oil was more effective against insect pest compared to the strain LE429. Thus, combined formulation of *L. enzymogenes* LE429 with neem oil should be a potential agent to control fungal diseases and insect pests.

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## References

- Abdel-Razek A.S., and S. Gowen. 2002. The Integrated Effect of the Nematode-Bacteria Complex and Neem Plant Extracts Against *Plutella xylostella* (L.) Larvae (Lepidoptera: Yponomeutidae) on Chinese Cabbage. Archives of Phytopathology and Plant Protection, Volume 35, Number 3:181-188(8).
- Amadioha, A.C. 2000. Controlling rice blast *in vitro* and *in vivo* with extract of *Azadirachta indica*. Crop Prot. 19:287-290.
- Cho, M.S., S.Y. Choi, T.W. Kim, C. Park, D.A. Kim, Y.R. Kim, S.M. Oh, S.W. Kim, Y.N. Youn, and Y.M. Yu. 2009. Insecticidal activity of Diamondback Moth, *Plutella xylostella* against *Bacillus thuringiensis* and Neem oil. The Korean Journal of Pesticide Science Vol. 13, No. 4:315-324.
- Crawford, D.L., J.M. Lynch, J.M. Whipps, and M.A. Ousley. 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Appl Environ Microbiol. 59:3899-905.
- Depieri, R.A., S.S. Martinez, and A.O. Menezes. 2005. Compatibility of the fungus *Beauveria bassiana* (Bals.) Vuill. (Deuteromycetes) with extracts of neem seeds and leaves, and the emulsible oil. Neotropical Entomology 34:601-606.
- Dey, R., K.K. Pal, D.M. Bhatt, and S.M. Chauhan. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol. Res. 159:371-394.
- El Shafie, H.A.F., and T. Basedow. 2003. The efficacy of different neem preparations for the control of insects damaging potatoes and eggplants in the Sudan. Crop Prot. 22:1015-1021.
- Elad, Y., I. Chet, and Y. Henis. 1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Can. J. Microbiol. 28:719-25.
- Folman, L.B., J. Postma, and J.A. Van Veen. 2003. Characterisation of *Lysobacter enzymogenes* (Christensen and Cook 1978) strain 3.1T8, a powerful antagonist of fungal diseases of cucumber. Microbiol. Res. 158:107-115.
- Handelsman, J., and E.V. Stabb. 1996. Biocontrol of soilborne plant pathogens. Plant Cell 8:1855-869.
- Hashizume, H., S. Hattori, M. Igarashi, and Y. Akamatsu. 2004. Tripropeptin E, a new tripropeptin group antibiotic produced by *Lysobacter* sp. BMK333-48F3. J. Antibiot. 57:394-399.
- Islam M.T., Y. Hashidoko, A. Deora, T. Ito, and S. Tahara. 2005. Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne peronosporomycetes. Appl. Environ. Microbiol. 71:3786-3796.
- Kim, P.I., and K.C. Chung. 2004. Production of an antifungal protein for control *Colletotrichum lagenarium* by *Bacillus amyloliquefaciens* MET0908. FEMS Microbiol. Lett. 234: 177-183.
- Ko, H.S., R.D. Jin, B.H. Krishnan, S.B. Lee, and K.Y. Kim. 2009. Biocontrol Ability of *Lysobacter antibioticus* HS124 Against Phytophthora Blight Is Mediated by the Production of 4-Hydroxyphenylacetic Acid and Several Lytic Enzymes. Curr Microbiol 59:608-615.
- Koul, O. 1999. Insect growth regulating and antifeedant effects of neem extracts and azadirachtin on two aphid species of ornamental plants. J. Biosci. 24:85-90.
- Kumar, P., H.M. Poehling, and C. Borgemeister. 2005. Effects of different application methods of neem against sweetpotato whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) on tomato plants. Journal of Applied Entomology 129:489-497.
- Lingappa, Y., and J.L. Lockwood. 1962. Chitin media for selective culture of actinomycetes. Phytopathology 52:317-323.
- Ma, L., X. Qiao, F. Gao, and B. Hao. 2001. Control of sweet pepper Fusarium wilt with compost extracts and its mechanism Chinese Journal of Applied & Environmental Biology. Chin. J. Appl. Environ. Biol. Vol. 7, no. 1:4-87.
- Mao, W., J.A. Lewis, P.K. Hebbar, and R.D. Lumsden. 1997. Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off caused by species of *Pythium* and *Fusarium*. Plant Diseases 81:450-455.
- Mao, W., R.D. Lumsden, J.A. Lewis, and P. K. Hebbar. 1998. Seed treatment using pre-infiltration and biocontrol agents to reduce damping-off of corn caused by species of *Pythium* and *Fusarium*. Plant Diseases 82:294-299.
- Mao, S., R.D. Jin, S.J. Lee, Y.W. Kim, K.H. Park, K.H. Cha, R.D. Park, and K.Y. Kim. 2006. Isolation and characterization of antifungal substance from *Burkholderia* sp. culture broth. Curr. Microbiol. 53:358-364.
- Mohan, G.T., and Gujar. 2001. Toxicity of *Bacillus thuringiensis* strains and commercial formulations to the diamondback moth, *Plutella xylostella* (L.). Crop Protection. 20:311-316.
- Nakayama, T., Y. Homma, Y. Hashidoko, J. Mizutani, and S. Tahara. 1999. Possible role xanthobaccins produced by *Stenotrophomonas* sp. strain SB-K88 in suppression of sugar beet damping-off disease. Appl. Environ. Microbiol. 65:4334-4339.
- Ortega, A.C. 1987. Insect Pests of Maize: A Guide for Field Identification. CIMMYT, Mexico, D.F., ISBN: 968-6127-07-0.

- O'Sullivan, J., J.E. McCullough, A.A. Tymiak, D.R. Kirsch, W.H. Trejo, and P.A. Principe. 1988. Lysobactin, a novel antibacterial agent produced by *Lysobacter* sp. J. Antibiot. 41:1740-1744.
- Patricia, M., and B. Larry. 2007. Rapid identification of gelatin and casein hydrolysis using TCA. Journal of Microbiological Methods 69:391-393.
- Patten, C.L., and B.R. Glick. 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68:3795-3801.
- Perez, L.A., and E.P. Cadapan. 1986. The efficacy of *Trichogramma* spp. as biological control agent against some rice insect pests. Philipp. Entomol., 6:463-470.
- Pemadasa, M.A. 1982. Effects of phenylacetic acid on abaxial and adaxial stomatal movements, and its interaction with abscisic acid. New Phytol. 92:21-30.
- Schaaf, O., A.P. Jarvis, S.A. Van der Esch, G. Giagnavoco, and N.J. Oldham. 2000. Rapid and sensitive analysis of azadirachtin, and related triterpenoids from neem (*Azadirachta indica*) by high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J. Chromatogr. 886: 89-97.
- Schirmbock, M., M. Lorito, Y.L. Wang, C.K. Hayes, I. Arisan-Atac, F. Scala, G.E. Harman, and C.P. Kubicek. 1994. Parallel formation and synergism of hydrolytic enzymes, and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. Appl. Environ. Microbiol. 60:4364-4370.
- Shen, S.S., O.H. Choi, S.M. Lee, and C.S. Park. 2002. *In vitro* and *in vivo* activities of a biocontrol agent, *Serratia plymuthica* A21-4, against *Phytophthora capsici*. Korean J Plant Pathol 18:221-224.
- Shipp, J.L., X. Hao, A.P. Papadopoulos, and M.R. Binns. 1998. Impact of western flower thrips (Thysanoptera: Thripidae) on growth, photosynthesis and productivity of greenhouse sweet pepper. Scientia Horticult 72:87-102.
- Shoda, M. 2000. Bacterial control of plant diseases. J. Biosci. Bioeng. 89:515-521.
- Ueeda, M., M. Kubota, and K. Nishi. 2006. Contribution of jasmonic acid to resistance against *Phytophthora* blight in *Capsicum annuum* cv. SCM334. Physiol. Mol. Plant Pathol. 67:149-154.
- Whipps, J.M., and D.R. Lumsden. 1991. Biological control of *Pythium* species. Biocontrol Science and Technology 1:75-90.
- Woo, S., V. Fogliano, F. Scala, and M. Lorito, 2002. Synergism between fungal enzymes and bacterial antibiotics may enhance biocontrol. Antonie Van Leeuwenhoek 81:353-356.
- Yedidia, I., N. Benhamou, Y. Kapulnik, and I. Chet. 2000. Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. Plant Physiol. Biochem. 38:863-873.

## ***Lysobacter enzymogenes* LE429와 Neem oil을 이용한 고추 병해의 생물학적 방제**

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근권토양으로부터 고추역병균을 포함한 다양한 식물 병원성 곰팡이에 대하여 항균활성이 강한 세균을 분리하였다. 이 세균은 16S rRNA gene서열 분석 결과 *Lysobacter enzymogenes*로 동정되었고 LE429로 명명 하였다. LE429는 chitinase,  $\beta$ -1, 3-glucanase, protease, gelatinase, lipase 및 항생물질과 같은 다양한 이차대사산물을 분비하였다. 항생물질은 diaon HP-20 및 sephadex LH-20 컬럼크로마토그래피 및 HPLC로 정제하여, GC-EI 및 GC-CI분석을 통하여 phenylacetic acid로 동정되었다. Field 실험에서 LE429의 고추 병해 억제 효과를 조사하기 위해 LE429배양액(CB), Neem oil 용액(NO), LE429배양액과 Neem oil 용액을 섞은 혼합액(CB+NO), 그리고 대조구로서 물(CON)을 각각 고추에 처리하였다. 고추의 수량구성요소는 일반적으로 CB 처리구가 가장 높았고, CB+NO, CON 그리고 NO 순서로 나타났다. CB 처리구에서 병원성 곰팡이는 강하게 억제 되었지만, 몇몇 해충이 발견되었다. NO 처리구에서는 해충은 발견 되지 않았지만, 병원성 곰팡이가 발견 되었다. 하지만, CB+NO 처리구에서 병원성 곰팡이 및 해충이 전혀 발견 되지 않았다. 결론적으로, 2차 대사산물을 생산하는 LE429와 Neem oil의 혼합액은 고추에 발생하는 병원성 곰팡이와 해충에 대한 좋은 생물학적 방제제가 될 수 있다고 사료된다.