*Korean J. Soil Sci. Fert.* 44(4), 650-656 (2011)

**Article**

# Investigation of Siderophore production and Antifungal activity against *Phytophthora capsici* as related to Iron (III) nutrition by *Lysobacter antibioticus* HS124

**Hyun Sun Ko, Hamisi Tindwa, Rong De Jin[1], Yong Seong Lee, Seong Hyun Hong, Hae Nam Hyun[2], Yi Nam[3], and Kil Yong Kim***

*Department of Biological Chemistry, Chonnam National University, Environment-Friendly Agriculture Research Center, Gwangju, 500-757, Korea, [1]Agricultural Environment and Resources Research Center, Jilin Academy of agricultural Sciences, Changchun 130033, China, [2]Major of plant resources & environment, Cheju National University, Cheju, 690-756, Korea, [3]Ansung Training Institute, National Agricultural Cooperative Federation, Ansung, Gongdoeup, Sinduri, 336-1, Korea*

***Lysobacter antibioticus* HS124 isolated from pepper rhizosphere soil produced catechol type siderophore. Purified siderophore by Diaion HP-20 and silica gel column chromatography showed several hydroxyl functional groups adjacent to benzene rings by analysis of $^{1}$H NMR spectroscopy. The strain HS124 showed different activities to suppress *Phytophthora capsici* with different concentrations of exogenous Fe (III) in minimal medium where antifungal activity with 100 µM Fe (III) was approximately 1.5 times higher than in absence of Fe (III). Bacterial population in this Fe (III)-amended medium was also highest with $8.9\times10^{8}$ CFU $ml^{-1}$ which also corresponded to the strongest siderophore activity. When grown in rich medium (minimal medium with N, $P_2O_5$, $K_2O$ and glucose), HS124 exhibited approximately 2 times stronger antifungal activity compared to minimal medium. In pot trials, treatments of bacterial culture grown in rich medium with (C1) or without (C2) 100 µM Fe (III) exhibited a high protection of pepper plants from disease, compared to medium only with (M1) or without (M2) 100 µM Fe (III). Especially, treatment C1 showed the best disease control effect of about 70 %. Thus, the strain HS124 should be recommended as a potential biocontrol agent against *P. capsici* in pepper.**

**Key words:** *Lysobacter antibioticus*, Siderophore, *Phytophthora capsici*, Antifungal activity, Biocontrol

## Introduction

A major advantage of using microorganisms in the biocontrol of phytopathogenic agents is that they can be more selective than chemicals and less harmful to the environment (Bolton et al., 1993) in addition to their rapid growth and aggressive colonization of the rhizosphere (Weller, 1988).

In aqueous, non-acidic, oxygenated environments such as the soil, iron which is an essential micronutrient required by all forms of life exists predominantly in its ferric ($Fe^{3+}$) form (Kraemer, 2005). Compounds of this predominant form of iron such as iron oxy-hydroxide are highly insoluble and, therefore, unavailable to microorganisms (Payne, 2005). To overcome iron deficiency, certain microorganisms secrete iron-binding molecules known as siderophores which serve as vehicles for the transport of iron (Fe III) into microbial cells (Loper and Henkels, 1997).

Production of siderophores gives the concerned organisms a better competitive power in relation to iron (Fe III) nutrition, and it is for this added advantage that siderophore-producing microbes have been effectively used as biocontrol agents against several pathogenic fungi. Siderophores produced by *Pseudomonas syringae* and *Pseudomonas viridiflava* (Bultreys and Gheysen, 2000), for example, played a vital role in the biocontrol of plant pathogens. Similarly siderophores produced by *Pseudomonas fluorescens* promoted plant growth via competition for iron (Loper and Henkels, 1999). Siderophore producing-*Pseudomonas* sp. was able

Received : July 20. 2011 Accepted : August 16. 2011
*Corresponding author : Phone: +82625302138
E-mail: kimkil@jnu.ac.kr

to promote growth of maize and suppress maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *F. graminearum* (Pal et al., 2001). Similarly, it has been demonstrated that members of the Genera *Bacillus*, *Pseudomonas* and *Kocuria* including *Bacillus firmus* D 4.1, *Pseudomonas aureofaciens* AR1 and *Kocuria rhizophila* 4(2.1.1) had strong antifungal activity against both *Fusarium oxysporum* and *Pyricularia oryzae*, the main causative agents of root rot and blast diseases of rice respectively (Chaiharn et al., 2009). Apparently, therefore, the ability of certain microbes to produce siderophore in addition to lytic enzymes plays a vital role in the biocontrol of plant pathogenic fungi.

Although several workers have reported that *Lysobacter* sp. produced several lytic enzymes such as chitinases and glucanases (Cho, 2007; Stepnaya et al., 2008) and several antibiotics like cetacandin A and B (Meyers et al., 1985), siderophore production is not yet known in *Lysobacter* sp. However, at least in our previous report (Ko et al., 2009) we indicated that the best biocontrol effects of *Lysobacter antibioticus* HS124 against *Phytophthora* blight were achieved by cultures of this strain grown in presence of exogenous Fe (III). The enhanced antifungal activity due to provision of exogenous Fe (III) could be linked to siderophore production and it is for this reason, therefore, that the current study aimed at characterizing siderophore produced by this strain and examining its effects on the antifungal activity of HS124 against *P. capsici* with different concentration of exogenous Fe (III).

## Materials and Methods

**Antagonistic microorganism and Siderophore assay** The bacterium, *Lysobacter antibioticus* HS124 (NCBI Accession No. FJ930928) having antagonistic activity to *Phytopthora capsici*, was used in this experiment. The 100 μl of HS124 culture previously grown in LB broth was inoculated on Chrome Azourol S (CAS) medium to examine the presence of siderophores (Schwyn and Neilands, 1987).

**Purification and identification of siderophore** Bacterial cultures were prepared by growing HS124 in LB medium (15 L) at 30℃ on a rotary shaker for 4 days. The culture broth so obtained was centrifuged at 6,000 rpm and 4℃ for 15 min. The supernatant was then acidified with concentrated HCl to pH 3 and extracted with ethyl acetate. The EtOAc soluble organic fraction was concentrated by a rotary evaporator to obtain the crude extract which was then dissolved in methanol and purified by Diaion HP-20 column chromatography (Mitsubishi Chemical Co., Japan) with a stepwise gradient of water-methanol (100:0~0:100, v/v). After all fractions of the elution were concentrated, each fraction dissolved in methanol was tested for presence of siderophores by both Arnow's and siderophore liquid assays (Schwyn and Neilands, 1987). The 100% methanol fraction showing siderophore activity was more purified by silica gel column chromatography (Kieselgel 60, 70-230 mesh, Merk, Darmstadt, Germany) with stepwise elution on an increasing concentration of EtOAc-MeOH (100:0, 50:50, 0:100, v/v). The fraction that showed positive siderophore reaction in 100% methanol was dried and purified further by the HPLC system with a $C^{18}$ reversed-phase column (Symmetry Prep $C^{18}$, 10 mm, 7.8× 300 mm, Waters). The mobile phase contained water: acetonitril (7:3, v/v) at a flow rate of 1.5 ml $min^{-1}$ and peaks were detected at 270 nm by an SPD-10 UV-VIS detector. Among the three detected, one peak showing strong siderophore activity was collected and dried for further experiments. The active compound dissolving DMSO-d6 was subjected to $^{1}H$ NMR (300MHz FT-NMR Spectrometer, Unity Plus 300, Varian) to determine hydroxyl functional group of siderophore.

**Bacterial growth, siderophore production, and antifungal activity in minimal medium with different concentration of Fe (III)** HS124 was inoculated on five treatment combinations of the minimal medium (water 1 L; crab shell powder 1.5 g; yeast extract 0.03 g; and pH 7.0) amended with $FeCl_3 \cdot 6H_2O$ to final concentrations of 0, 50, 100, 150, 200 μM respectively and incubated for five days at 27℃. At the end of the incubation period, the bacterial population in each treatment combination was counted by the dilution plate count technique on the LB agar medium. The siderophore activity was measured by inoculating 100 μl of the supernatant from each treatment combination on CAS medium for 1 day followed by measuring the size of the halo zone. To determine the antifungal activity, the PDA medium was mixed with the supernatant from each treatment combination which was either sterilized for 15 minutes at 121℃ prior to mixing or non-sterilized to a final concentration of 30%, respectively. Immediately after media became solid on (9×9 cm), petri dishes, one plug (6 mm) of *P. capsici*, which was previously grown on

PDA for 7 days at 27℃, was inoculated. Petri-dishes with un-inoculated medium were used as control treatments. After 5 days of incubation at 27℃, growth of *P. capsici* was measured by comparison with control plates. Three replicates were run simultaneously. The antifungal activity was taken as mean of the percentage growth inhibition calculated by the following formula: Inhibition rate (%) = [(A−B)/A×100], whereas A = mycelial growth in control, B = mycelial growth on medium amended with the bacterial supernatant.

**Antifungal activity in rich medium** HS124 was grown in rich medium (which contained glucose 2 g; N 0.42 g; $P_2O_5$ 0.34 g; and $K_2O$ 0.34 g per 1 L in addition to the contents of the minimal medium at pH 7.0) with (C1) or without (C2) 27 mg (100 μM) $FeCl_3 \cdot 6H_2O$. Before the resultant cultures were added to the pepper rhizosphere, both C1 and C2 were tested for antibiotic activity against *P. capsici*. Un-inoculated media with (M1) or without (M2) the additional 27 mg $FeCl_3 \cdot 6H_2O$ were used as controls. After 5 days of incubation, the supernatant from each of the treatment combinations was either sterilized at 121℃ for 15 min or non-sterilized, and then mixed with PDA medium to a final concentration of 15, 30 and 50%, respectively. One plug of *P. capsici* was inoculated to each of the treatment combinations as described above and incubated for 5 days at 27℃. Three replicates were run simultaneously. The percentage of growth inhibition was measured as described above.

**Pathogen inoculum** *P. capsici* (KACC 40483) was grown on V8 juice agar medium at 30℃ and incubated for 5 days. The fungal media were then cut into pieces which were flooded with sterile de-ionized water. The de-ionized water was replaced on a daily basis all through the incubation period. The continuous incubation of the flooded samples was performed under fluorescent light for 5 days at 30℃ to produce sporangia and then chilled at 4℃ for 30 min to release zoospores. The zoospore suspension was filtered by sterile cheesecloth and diluted with sterile water to a concentration of $1\times10^6$ zoospore $ml^{-1}$ (Meyers et al., 1985).

**Plant growth condition and Disease incidence** Pepper seeds (*Capsicum annuum* L.) were sown in 3×3 cm plastic cell plug trays filled with commercial grade bed soil (Bio bed soil Ⅰ, Heong Nong Seed Co. South Korea). Four weeks after sowing, pepper seedlings were transplanted to pots containing 600 g of non sterilized-soil mixture (soil: sand: vermiculite, 2:1:1, v:v:v) and maintained at 24℃ in an artificially illuminated room (12,000 lux at plant height) set at a 16 hr photoperiod. At 2, 3, 4 and 5 weeks after planting, each pot was amended with 30 ml of (C1), (C2), (M1) or (M2) respectively. Six weeks after transplanting, 30 ml of zoospore suspension of *P. capsici* ($1\times10^6$ zoospores $ml^{-1}$) were poured into pepper rhizosphere soils of each treatment combination. Plants were harvested at 8 days after infection with *P. capsici* zoospores. Disease incidence was determined by counting diseased plants, expressing it as a percentage of the total plants in respective treatments.

## Results

**Detection of siderophore production** For the simple detection of siderophore production, 100 μl of *Lysobacter antibioticus* HS124 culture was loaded on CAS medium. After 24 h incubation, a change in color from blue to orange was observed attributed to formation of siderophore-Fe (III) complex (Fig. 1).

**Identification of siderophore** Following Diaion HP-20 and silica gel column chromatography, the siderophore active fraction was purified by HPLC analysis and each of the detected peaks was tested by siderophore liquid assay. One peak showing the strongest siderophore activity was obtained from a distinct fraction of Rt 9.15 (data not shown). When $^1H$ NMR spectroscopy was performed for identification of siderophore, spectrum showed several

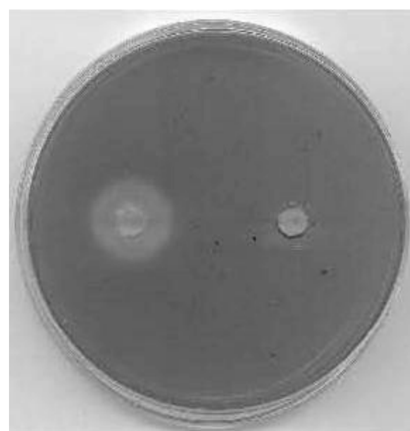

**Fig. 1. Orange halo zone due to siderophore production from *L. antibioticus* HS124 (left) and only medium as control (right) on Crome Azourol S agar medium incubated at 30℃ for 1 day.**

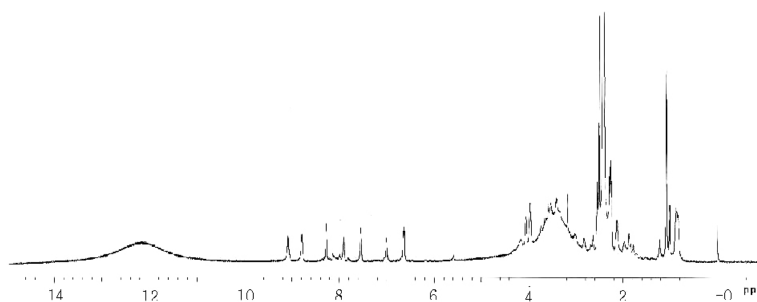


**Fig. 2. $^{1}$H NMR spectrum of extracted siderophore from *L. antibioticus* HS124.**

**Table 1. Change in *L. antibioticus* HS124 population and siderophore activity in the minimal medium with different concentrations of Fe (III) incubated at 30℃ for 5 days.**

| Fe (Ⅲ) concentraion | Bacterial population (CFU mL$^{-1}$)$^{†}$ | Siderophore activity$^{‡}$ |
|---|---|---|
| Fe (Ⅲ) 0 μM | $1.1 \times 10^8$ | +++ |
| Fe (Ⅲ) 50 μM | $7.9 \times 10^8$ | +++ |
| Fe (Ⅲ) 100 μM | $8.9 \times 10^8$ | +++ |
| Fe (Ⅲ) 150 μM | $3.5 \times 10^8$ | ++ |
| Fe (Ⅲ) 200 μM | $3.2 \times 10^8$ | ± |

†counted on LB agar medium by dilution plate method.
‡measured by size of halo zone and reported as (±) < 1 mm, (++) between 3 and 4 mm, (+++) < 7 mm.

hydroxyl groups at around 12 ppm and 2 benzene rings at around 8 ppm, meaning that this structure matched with the basic siderophore structure (Fig. 2).

**Bacterial growth, siderophore production, and antifungal activity in minimal medium with different concentration of Fe (III)** Bacterial growth, siderophore production and antifungal activity of strain HS124 were evaluated at different concentrations (ranging from 0 to 200 μM) of exogenous Fe (III). As shown in Table 1, the bacterial population increased with increasing concentrations of exogenous iron (III). The population size reached a peak of $8.9 \times 10^8$ CFU ml$^{-1}$ at 100 μM but increasing the concentration to values beyond 100 μM caused a sharp decline in the population sizes with the lowest value of $3.2 \times 10^8$ CFU ml$^{-1}$ recorded at 200 μM. the siderophore activity followed a somewhat similar trend (Table 1) with strongest activities observed at iron (III) concentrations of between 0 and 100 μM. A steady decrease of siderophore activity was observed at iron (III) concentrations higher than the 100 μM. To test antifungal activity of each treatment combination against *P. capsici*, the respective supernatants were added into PDA medium to a final proportion of 30%. A stepwise increase of the iron (III) concentration to 100 μM caused a corresponding increase in antifungal activities in both cases- sterilized and non sterilized (Fig. 3). However, increasing the concentration of the exogenous iron to levels beyond 100 μM resulted in sudden decrease of activity. It was observed further that the antifungal activity against *P. capsici* was markedly reduced by sterilization. Thus, at any iron (III) concentration the antifungal activity of the non-sterilized treatment was markedly higher than of sterilized.

**Antifungal activity in rich medium** HS124 was gown in rich medium with (C1) or without (C2) Fe (III) for 5 days, and then tested for antifungal activity. The antifungal activities of C1 and C2 were always stronger in non-sterilized than those of sterilized condition (Fig. 4). Increasing the concentration of exogenous iron resulted in an increase in the antifungal activity owing to the enhanced capacity to produce siderophores. As a result, *P. capsici* growth was completely inhibited in the treatment combination that was amended with the highest (50%) iron (III) concentration.

**Biocontrol effect by *L. antibioticus* HS124** In this experiment, the four different treatments namely Culture with (C1) or without (C2) iron (III) and un-inoculated medium with (M1) or Without (M2) iron (III) were applied to the rhizosphere of potted soil grown to pepper plants. Plants that received the un-inoculated treatments (M1 & M2) had succumbed to wilt and rot of the stems within 5

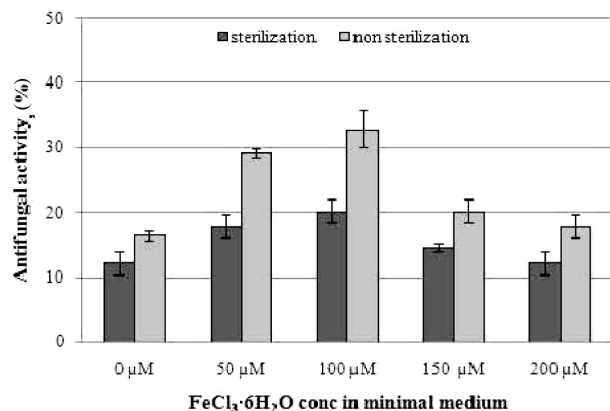


**Fig. 3. Antifungal activities of sterilized and non-sterilized bacterial cultures (*L. antibioticus* HS124) with different concentrations of Fe (III) in minimal medium against *P. capsici* measured after 5 days of incubation at 30℃. The culture of *L. antibioticus* HS124 in each treatment was made to a final concentration of 50% in PDA and incubated at 30℃ for 5 days, prior to the addition of a plug of *P. capsici*.**

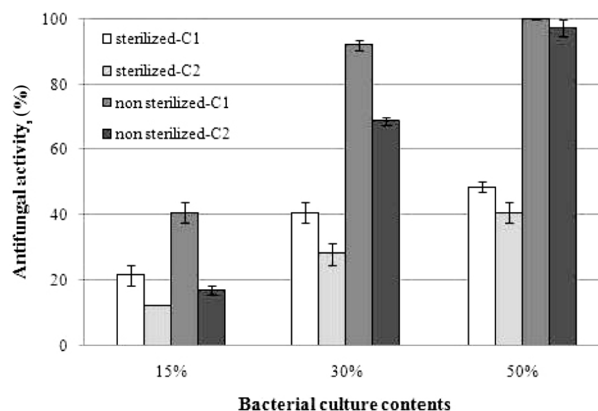


**Fig. 4. Antifungal activities of sterilized and non-sterilized bacterial culture with (C1) or without (C2) the 100 μM Fe (III) in rich medium against *P. capsici*. The culture of *L. antibioticus* HS124 in each treatment was made to a final concentration of 15, 30, and 50% in PDA and incubated at 30℃ for 5 days, prior to the addition of a plug of *P. capsici*.**

**Fig. 5. Growth of pepper plants as infected with *P. capsici* after treatments of each bacterial culture grown in rich medium with (C1) or without (C2) 100 μM Fe (III) and only medium with (M1) or without (M2) 100 μM Fe (III) were used as controls.**

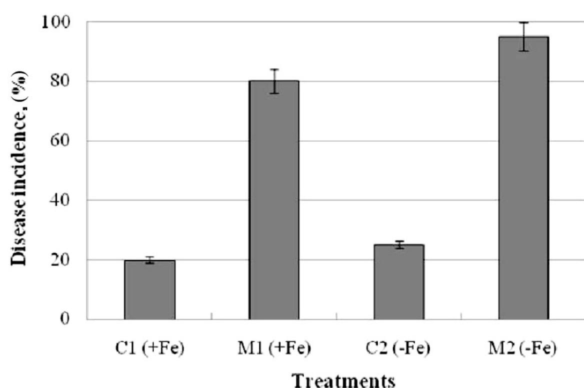


**Fig. 6. Disease incidence due to infestation by *P. capsici* after treatments of each bacterial culture grown in rich medium with (C1) or without (C2) 100 μM Fe (III) and only medium with (M1) or without (M2) 100 μM Fe (III) as controls. Bars represent standard error.**

days after infection with symptoms initially appearing on the stems and then suddenly spreading over the entire plant. However, the plants that received the bacterial inoculation (C1 & C2) remained healthy (Figure 5). Disease incidence by *P. capsici* in pepper plants was lowest (only 20%) in (C1) treatment followed by (C2), (M1), and (M2) in that order (Fig. 6). In both cases, treatments that received iron (III) performed much well than those which did not receive the additional exogenous iron.

## Discussion

This work is a continuation of a series of experiments aimed at examining the biocontrol ability of *Lysobacter antibioticus* HS124 against *phytophthora* blight. We reported previously (Ko et al, 2009) that the broad-spectrum of antifungal activity demonstrated by HS124 could be attributed to its ability to produce several lytic enzymes such as chitinase and β-1,3 glucanase and secondary

metabolites such as 4-hydroxyphenylacetic acid. In the current report we focused our attention to the characterization of siderophore produced by HS124 and subsequently examine its contribution to the overall biocontrol ability of this strain against *Phytophthora capsici*.

The ability to produce siderophores as linked to iron nutrition is known to occur in an array of microorganisms. Other workers have reported siderophore production in Pseudomonads (Meyer and Abdallah, 1978), *Streptomycetes*, *Lactobacillus*, *Staphylococcus* (Nishio and Ishida, 1989) and *Azospirillum lipoferum* (Samir et al., 1992) to mention but a few. In our results, formation of orange halo zones around an inoculation of HS124 on CAS medium indicated the production of siderophores (Fig. 1). To the best of our knowledge, this is the first report on production of siderophores by a *Lysobacter* strain. The siderophore produced by HS124 belonged to catechol type on the basis of the fact that the hydroxyl groups were adjacent to benzene rings bound to Fe (III) as shown by $^{1}$H NMR spectroscopy (Fig. 2).

The siderophore activity (and production) in HS124 increased over a relatively wide range of iron concentrations reaching a peak at 100 μM Fe (III), followed by a sharp decline at concentrations beyond 100 μM. The observed pattern is generally consistent with earlier reports that gradual repression of siderophore production was observed with increasing concentrations (0 to 10 μM) of iron (III) in *Pseudomonas syringae* (Bultreys and gheysen, 2000), and that total repression of siderophore production by the same species was observed at iron (III) concentration of 100 μM (Laine et al., 1996). However, our findings suggest that Fe (III) requisition by HS124 was considerably higher than those of other siderophore producing bacteria. In this regard, we found interesting relationship between Fe (III) concentration and antifungal activity against *P. capsici*. As shown in Fig. 3, antifungal activity of HS124 supplemented with 100 μM Fe (III) showed approximately 1.5 times higher values compared to absence of Fe (III) against *P. capsici*. These results suggested that the presence of 100 μM of Fe (III) was necessary for optimal microbial growth and production of secondary metabolites including siderophores. This finding is similar to a report that sufficient iron was necessary for *Pseudornonas fluorescens* Strain CHAO to effectively suppress the effects of *Thielaviopsis basicola*-a causative agent of black root rot in tobacco (Keel et al., 1989), and that the stimulatory effects of Fe (III) on production of some biocontrol secondary metabolites such as cyanide and phenazine by *Pseudomonas chlororaphis* PCL1391 (Van Rij et al., 2004).

When the antifungal activity of the bacterial culture grown in minimal medium with 100 μM Fe (III) (Fig. 3) was compared to that of bacterial culture grown in rich medium (Fig. 4), the antifungal activity of the latter was double as much as that of the former. This indicated that the synthesis of antifungal metabolites depends on the amount and type of medium constituents. In fact, it has been shown that the carbon and other nutrient sources strongly affect antibiotic production in different organisms, and glucose in particular, increased antibiotic production potential in the rich medium (Cruz et al., 1999; Vilches et al., 1990).

The pathogen, *P. capsici*, has a broad host range. It is known to attack tomato, eggplant, cucumber, watermelon, pumpkin, squash, cocoa, macadamia, and pepper (Kunimoto et al., 1976; Ristaino, 1990). To control *Phytophthora* blight disease, various soil bacteria have been widely used and only a few have been successfully applied in the field. In our current report, protective effects of HS124 against a pathogenic strain *P. capcisi* were investigated on the pepper plants. There were significant differences in height and fresh weights between HS124 containing treatments (C1, C2) and un-inoculated (only medium) treatments (M1, M2) observed 8 days after infection (data not shown). C1 and C2 treatments greatly protected plants from *P. capsici* compared to M1 and M2 treatments (Fig. 5 and 6). This is consistent with reports that the enhanced plant growth caused by siderophore producing bacteria was accompanied by a corresponding reduction in the pathogen populations on the root (Meziane et al., 2005) and that the production of siderophores by the antagonist appeared to deprive the pathogen of iron nutrition and consequently inhibited basidiospore germination by its culture supernatants (Macagnan et al., 2008).

Conclusively, the antagonist bacterium HS124 has shown strong siderophore and antifungal activities thus, exhibiting its effective biocontrol ability against *P. capsici* infecting pepper plants. This was especially obvious when the antagonistic bacterium was grown in rich medium (minimal medium plus N, $P_2O_5$, $K_2O$ and glucose). HS124 may, therefore, be a useful biocontrol agent against phytopathogenic fungal infection such as that caused by *P. capsici*. Further studies are underway to evaluate the biocontrol efficacy of this strain in fields infected with various fungal pathogens.

## References

Arnow, L.E. 1937. Colorimetric determination of the compounds of 3,4-dihydroxyphenylalanine-tyrosine mixture. J Biol Chem 118:531-537.

Bolton, H., J.K. Fredrickson, and L.F. Elliott. 1993. Microbial ecology of the rhizosphere. In: Metting, F.B. (Ed). Soil microbial ecology: Applications in Agricultural and Environmental Management. Marcel Dekker New York. p. 27-63.

Bultreys, A. and I. Gheysen. 2000. Production and comparison of peptide siderophores from strains of distantly related pathovars of *Pseudomonas syringae* and *Pseudomonas viridiflava* LMG 2352. Appl Environ Microbiol 66:325-331.

Chaiharn, M., S. Chunhaleuchanon, and S. Lumyong. 2009. Screening siderophore producing bacteria as potential biological control agent for fungal rice pathogens in Thailand. World J. Microbiol & Biotechnol 25(11):1919-1928.

Cruz, R., M.E. Ariasand, and J. Soliveri. 1999. Nutritional requirement for the production of pyrazoloisoquinolinone antibiotics by *Streptomyces griseocirneus*. NCIMB 40447. Appl. Microbiol. Biotechnol. 53:115-119.

Csaky, T. 1948. On the estimation of bound hydroxylamine. Acta. Chemica. Scandinavica. 2:450-454.

Keel, C., C. Voisard, C.H. Berling, G. Kahr, and G. Defago. 1989. Iron sufficiency, a prerequisite for the suppression of tobacco black root rot by *Pseudomonas fluorescens* strain CHA0 under gnotobiotic conditions. Phytopathology 79:584-589.

Ko, H.S., R.D. Jin, H.B. 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-Hydroxylacetic acid and several lytic enzymes. Curr. Microbiol. 59:608-615.

Kunimoto, R,K., M. Aragaki, J.E. Hunter, and W.H. Ko. 1976. *Phytophthora capsici*, corrected name for the cause of *Phytophthora* blight of macadamia racemes. Phytopathology 66:546-548.

Kraemer, S.M. 2005. Iron oxide dissolution and solubility in the presence of siderophores. Aquatic Science 66:3-18.

Laine, M.H., M.T. Karwoski, L.B. Raaska, and T.M. Mattila. 1996. Antimicrobial activity of *Pseudomonas* spp. against food poisoning bacteria and moulds. Lett. Appl. Microbiol. 22:214-218.

Cho, M.Y. 2007. Biocontrol of *Phytophthora* blight (*Phytophthora capsici*) in pepper by *Lysobacter enzymogenes* LE429. A Master's thesis, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, Republic of Korea pp. 50.

Loper, J.E., and M.D. Henkels. 1997. Availability of iron to *Pseudomonas fluorescens* in rhizosphere and bulk soil evaluated with an ice nucleation reporter gene. Appl. Environ. Microbiol. 63:99-105.

Loper, J.E., and M.D. Henkels. 1999. Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. Appl. Environ. Microbiol. 65:5357-5363.

Macagnan, D., R.S. Romeiro, A.W.V. Pomella, and J.T. deSouza. 2008. Production of lytic enzymes and siderophores, and inhibition of germination of basidiospores of *Moniliophthora* (*ex Crinipellis*) *perniciosa* by phylloplane actinomycetes. Biol. Control 47:309-314.

Meyer, J.M. and M.A. Abdallah. 1978. The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physical-chemical properties. J. Gen. Microbiol. 107:319-328.

Meyers, D., R. Cooper, L. Dean, J.H. Johnson, D.S. Slusarchyk, W.H. Trejo, and P.D. Singh. 1985. Catacandins, novel anticandidal antibiotics of bacterial origin. J. Antibiot. 38:1642-1648.

Meziane, H., I. Van Der Sluis, L.C. Van Loon, M. Hofte, and P.A.H.M Bakker. 2005. Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. Mol. Plant Pathol. 6:177-185.

Nishio, T. and Y. Ishida. 1989. Distribution of iron solubilizing bacteria in sediment of a small lagoon. Nippon Suisan Gakkaishi 55(11):1955-1960.

Pal, K.K., K.V. Tilak, A.K. Saxena, R. Dey, and C.S. Singh. 2001. Suppression of maize root disease caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *F. graminearum* by plant growth promoting rhizobacteria. Microbiol Res.156:209-223.

Payne, S.M. 1994. Detection, isolation, and characterization of siderophore. Method Enzymol. 235:329-344.

Ristaino, J.B. 1990. Intra specific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. Phytopathology 80:1253-1259.

Samir, S., K.Vaidehi, and D. Anjana. 1992. Isolation and characterization of siderophore, with antimicrobial activity, from *Azospirillum lipoferum* M. Curr Microbiol 25:347-351.

Schwyn, B. and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160:47-56.

Stepnaya, O.A., I.M. Tsfasman, I.A. Chaika, T.A. Muranova, and I.S. Kulaev. 2008. Extracellular yeast-lytic enzymes of the bacterium *Lysobacter* sp. XL 1. Biochemistry 73:310-314.

Van Rij, E.T., M. Wesselink, T.F.C. Chin-A-Woeng, G.V. Bloemberg, and B.J.J. Lugtenberg. 2004. Influence of environmental conditions on the production of phenazine-1-carboxamide by *Pseudomonas chlororaphis* PCL1391. The American Phytopathological Society 17:557-566.

Vilches, C., C. Mendez, C. Hardission, and J.A. Salas. 1990. Biosynthesis of oleandomycin by *Streptomyces antibioticus*: influence of nutritional conditions and development of resistance. J. Gen. Microbiol. 136:1447-1454.

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