African Journal of Biotechnology Vol. 10(36), pp. 6920-6927, 18 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.260
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Induction of resistance in cucumber against seedling damping-off by plant growth-promoting rhizobacteria (PGPR) *Bacillus megaterium* strain L8

Jian-gen Liang[1], Rong-xiang Tao[1]*, Zhong-na Hao[1], Lian-ping Wang[1] and Xin Zhang[2]

[1]Institute of Plant and Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, PR China.
[2]School of Forestry and Biotechnology,Zhejiang Agricultural and Forestry University, Lin,an 311300, PR China.

Accepted 17 June, 2011

Bacillus megaterium* L8, a plant growth-promoting rhizobacterium (PGPR), was isolated and evaluated for its ability to induce resistance in cucumber against seedling damping-off caused by *Pythium aphanidermatum*. Root-splitting challenge experiments showed that L8 treatment of cucumber roots more effectively suppressed seedling damping-off than did the control as judged by seedling survival rate (83.45 versus 31.68% at 28 days), indicating that strain L8 is capable of inducing systemic disease resistance in cucumber. To explore the potential mechanism underlying the induced systemic resistance (ISR) mediated by L8, the expression profile of several plant defense-related enzymes: superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) were monitored in the roots treated with L8 or *P. aphanidermatum* and untreated leaves in a time course of 13 days. Levels of SOD, POD, CAT, PPO and PAL activities in the treated roots and untreated leaves of cucumber seedlings were all significantly higher as compared with the control and respectively peaked in the roots at 3, 3, 5, 5 and 5 days and correspondingly in the leaves at 5, 3, 5, 5 and 5 days post-inoculation. The enhanced expression patterns of the above enzymes following L8 or *P. aphanidermatum* treatment suggested that systemic induction of plant defense enzymes by L8 might account for its ability to provide effective protection for cucumber from seedling damping-off caused by the soil-borne fungal pathogen *P. aphanidermatum.

Key words: Plant growth-promoting rhizobacteria (PGPR), defense enzymes, damping-off of cucumber seedlings, induced resistance.

INTRODUCTION

Plant enzymes are involved in defense reactions against plant pathogens. These include oxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and polyphenol oxidase (PPO), which catalyse the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure (Avdiushko et al., 1993). Other enzymes such as phenylalanine ammonia-lyase (PAL) are involved in phytoalexin or phenolic compound biosynthesis (Bashan et al., 1985; Beaudoin-Eagan and Thorpe, 1985). Such enzymes have been correlated with defense against pathogens in several plants, including tobacco, tomato, cucumber and rice (Beaudoin-Eagan, 1985; Goy et al., 1992; Bashan et al., 1985; Rajappan et al., 1995). These plant enzymes have long been thought to play an important role in the plant defense.

Of the numerous biocontrol agents identified since 1980s, one interesting class of beneficial microorganisms known as plant growth-promoting rhizobacteria (PGPR) has attracted considerable attentions of the biocontrol community with the findings in 1991 since PGPR are capable of inducing systemic resistance in cucumber and carnation (Van Peer et al., 1991; Wei et al., 1991). Subsequent studies have showed the involvement of a

*Corresponding author. E-mail: ljg422@ eyou.com. Tel: +86-571-86419027. Fax: +86-571-86404266.

Abbreviations: SOD, Superoxide dismutase; **POD,** peroxidase; **CAT,** catalase; **PPO,** polyphenol oxidase; **PAL,** phenylalanine ammonia-lyase; **PGPR,** plant growth-promoting rhizobacteria.

variety of PGPR and other biocontrol agents in induced systemic resistance (ISR) in several crops against a wide spectrum of pathogens including fungi, bacteria, viruses and nematode (Van Loon et al., 1998; Chen et al., 1998; Curtis et al., 1997; Maurhofer et al., 1994; Wei et al., 1991; Whipps, 2001). This phenomenon, termed ISR (Kloepper et al., 1992), markedly differs from systemic acquired resistance (van Loon, 1997). However, little work has been reported on how these rhizobacteria may affect the induction of defense enzymes activity in cucumber.

Recently, Jetiyanon et al. (1997) showed that *Bacillus pumilus* strain SE49 enhanced lignification and total POD but not chitinase activity. Moreover, *Bacillus subtilis* AF1 induced an increase in PAL and POD activities in pigeonpea (Podile and Laxmi, 1998). Strains of PGPR have also been shown to induce systemic resistance in cucum-ber to a root pathogen, *Pythium aphanidermatum* (Chen et al., 1998; Zhou et al., 1994). In previous studies, with split root systems, *Pseudomonas corrugata* strain13 and *Pseudomonas aureofaciens* strain 63-28 were shown to systemically suppress *P. aphanidermatum* on cucumber roots (Chen et al., 1998; Chen et al., 1999). However, little is known about the induction of resistance in cucumber against seedling damping-off by plant growth-promoting rhizobacteria (PGPR) *Bacillus megaterium*. In this study, we evaluated the ability of a novel PGPR *B. megaterium* L8, using the root-split system, to induce systemic resistance in cucumber against seedling damping-off caused by the fungal pathogen *P. aphanidermatum*; the objective was to determine whether this induction was systemic. In order to answer this question, enzyme activities in leaves except for roots were also examined which were separated from the inducing treatment. And here we provided data showing that L8 is capable of inducing systemic resistance in cucumber against seedling damping-off by *P. aphanidermatumin*. In addition, to test our hypothesis that activation of plant defense enzymes is associated with L8-mediated induced systemic resistance (ISR) in cucumber, the enzymatic activity profiles in a time course were monitored for several plant defense-related enzymes (SOD, POD, CAT, PPO and PAL) in the roots and leaves after treatment.

MATERIALS AND METHODS

Isolation and preparation of the PGPR *B. megaterium* strain L8

The L8 strain was isolated from a vegetable garden soil of multi-year cucumber monoculture at HuaJiaChi campus of Zhejiang University. Both pot tests and small field trials showed that L8 markedly enhanced cucumber growth. Based on the routine procedure for bacteria characterization combined with 16S rDNA sequence analysis, L8 was identified as *B. megaterium*. Cell suspensions of the strain was prepared by inoculating it into YPG liquid medium (yeast extract 5 g, glucose 5 g, tryptone 5 g, NaCl 1 g, water 1000 ml) and cultivated on a shaker (150 rpm) at 28°C for 48 h. Bacterial cells were washed by centrifuging at 5000 *g* for 10 min at 4°C, pellets were washed and re-suspended in 0.1 M $MgSO_4$ to a concentration of 10^8 cfu/ml for inoculation via plating approach.

Fungal isolate

The pathogenic P. *aphanidermatum* isolate stain 8 used for challenge inoculation was maintained on potato dextrose agar (PDA) at room temperature for short-term storage in our laboratory. The stored *P. aphanidermatum* was reactivated on a *Pythium* selective medium (Van Der Plaats-Niterink, 1981) and inoculated sequentially into a flask containing sterilized bran mash medium to grow at 25°C for 10 to 15 days before its use for challenge inoculation.

Split-root assay

The assay was performed according to a procedure of Press et al. (1997). The root of individual healthy cucumber seedlings with fully developed cotyledon was separated into two halves and planted into two adjacent small plastic pots; one pot filled with sterilized soil drenched with 30 ml L8 suspension at a concentration of 3×10^8 cfu/ml and the other filled with infested soil (*P. aphanidermatum:* soil (w/w)= 1: 20). For the control, the entire root of each cucumber seedling was planted into each pot filled with *P. aphanidermatum*-infested soil. The general growth feature and seedling survival rate were recorded.

Experimental design

Dynamics of enzyme activity in cucumber roots and leaves were inoculated with PGPR or pathogen. Seeds of cultivar XinTai MiCi were surface sterilized with 1% sodium hypochlorite and planted in plastic pots (height 25, top 15 and base 20 cm in diameter) filled with twice-autoclaved fertile garden soils. The seedlings were fertilized with liquid nutrient solution every other day after planting. There were four treatments: (1) P. *aphanidermatum* inoculation plus root-drenching with L8 suspension (using a split-root assay); (2) *P. aphanidermatum* treatment alone; (3) root-drenching with L8 suspension alone; (4) H_2O treatment (CK). The experiment was a factorial design in a randomized complete block. Cucumber plants were grown in a greenhouse bench at a temperature of 25°C. Samples of respective roots and leaves were collected post-inoculated at 1, 3, 5, 7, 9, 11 and 13 days and were used for the measurement of activity of several defense-related enzymes. Each treatment had three repeats. A time course was employed to express the enzymatic activities on different days after treatment.

Enzyme activity assay

Fresh cucumber roots and leaves were washed in running tap water after sampling and homogenized with liquid nitrogen in a mortar and pestle. The homogenized tissue was rinsed with the same volume of 50 mM sodium phosphate buffer (pH 6.8) at 4°C and filtered into a centrifuge tube. The tissue extracts were centrifuged at 12000 *g* for 15 min at 4°C. The supernatant to be used for the enzymatic activity assay was transferred to 1.5 ml vial and stored at -20°C. A colorimetric assay for enzymatic activity was performed with a native spectrophotometer.

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was determined using an NBT method, as described previously by Zhou et al. (2003). The root and leaf tissue extracts were diluted 10-fold with the

Table 1. Induced resistance by the PGPR strain L8 to seedling damping-off of cucumber.

Treatment	Total number	Seedling survival rate (%)		Control efficacy (%)	
		14 days	28 days	14 days	28 days
L8 (via root-split)	50.00	86.78 ± 0.24[a]	83.45 ± 0.13[a]	79.13	75.78
CK	50.00	36.65 ± 0.16[b]	31.68 ± 0.09[b]	–	–

There were two treatments: (1) *P. aphanidermatum* inoculation plus root-drenching with L8 suspension (using a split-root assay); (2) *P. aphanidermatum* treatment alone (CK). Values followed by the same letter (s) were not significantly different at $P < 0.05$ according to LSD test. Relative bio-control efficacy = (Seedling survival rate in each treatment-CK)/(1-CK).

homogenization buffer. 20 µl of the diluted extracts were added to 3 ml of 50 mM sodium phosphate buffer (pH 6.8 and mixed with 2.7 ml 13 mM methionine; 100 µl 75 µM nitroblue tetrazolium and 100 µl 0.1 µM disodium ethylenediamine tetraacetate in the mixture). The reaction was started by the addition of 100 µl riboflavin to the mixture under 4000 Lx candescent lamp and the initial rate of increase in absorbency at λ 560 nm was measured over 15 min. The OD_{560} was measured with a 7230 spectrophotometer and each experiment contained 3 repeats and the amount of SOD enzyme that inhibited 50% of NBT reduction was defined as one enzyme unit (U).

Peroxidase (POD)

Peroxidase (POD) was measured by the method of Moerschbacher et al. (1998). The root and leaf tissue extracts were diluted 5-fold with the homogenization buffer. 40 µl of the diluted extracts were added to 2.91 ml of 50 mM sodium phosphate buffer (pH 6.8 and mixed with 100 µl guaiacol 20 mM in the mixture). The reaction was started by the addition of 40 µl 40 mM H_2O_2 to the mixture and the initial rate of increase in absorbency at λ 470 nm was measured over 3 min. Absorbance at 470 nm was measured using a 7230 spectrophotometer and each experiment had 3 repeats. One unit of POD activity was defined as the amount of enzyme that increases the OD470 value by 0.01.

Catase (CAT)

CAT was determined according to the procedure of Zhou (1995). Briefly, 10 ml of the crude enzyme preparation (10 ml of distilled H_2O as control) was added to a 50 ml flask, followed by addition of 5 ml of 55 mM H_2O_2 and placed at 30°C for 10 min. The reaction was stopped by addition of 5 ml of 1.8 mM H_2SO_4, followed by addition of 1 ml of 20% KI first and then 2 to 3 drops of ammonium molybdate and starch indicator. Subsequent titration was conducted using $Na_2S_2O_3$ (0.02 M) until the solution turned blue. The amount of $Na_2S_2O_3$ used was recorded and the amount of the hydrolyzed H_2O_2 by CAT was calculated based on the different consumption of the $Na_2S_2O_3$ used for the sample and the control. The activity of CAT was defined as the number of micromole of H_2O_2 by 1 g fresh roots within 1 min.

Phenylalanine ammonia-lyase (PAL)

PAL activity was determined as described by Mozzetti (1995). Briefly, 0.5 g fresh roots was cut into small pieces and ground in a pre-chilled mortar with 5 ml of pre-chilled 50 mM PBS buffer (pH 8.8, containing 5 mM mercaptoethanol and 1 mM EDTA) and 0.025 g polyethylene pyrrole alkane ketone (PVP) and the samples was centrifuged at 4°C (12000 *g*, 20 min) and the supernatant collected. PAL activity was measured based on OD_{290} using a spectrophotometer (Daojin UV210). Each experiment had 3 repeats. One unit of enzyme is defined as the amount of enzyme that increases the OD_{290} value by 0.01.

Polyphenoloxidase (PPO)

PPO was determined according to the method of Zhu (1990). 200 ml of the root and leaf extracts used for PPO (catechol oxidase) assay were mixed with 250 µl of 50 mM sodium phosphate buffer. The rate of increase in absorbency at λ 525 nm was measured for 2 min after the addition of 500 µl 0.1M catechol. The OD_{525} was measured using a 7230 spectrophotometer. Each sample had 3 repeats. One unit of enzyme is defined as the amount of enzyme that increases the OD525 value by 0.01.

Data analyze

All analyses were performed using SPSS10 (SPSS Inc. Headquarters, 233 S. Wacker Drive, 11th floor Chicago, Illinois 60606). The general linear model (GLM) procedure was used to test if PGPR or pathogen affected the enzymatic activity in cucumber roots at different days after bacterization. The characteristic of enzyme dynamics was expressed as activity over time and a repeated measures analysis was used to analyze activities of enzymes over time. Means of the enzymatic activity for each treatment were separated with a least significant difference (LSD, $P < 0.05$) multiple comparion test.

RESUTS

Systemic induction of resistance by *B. megaterium* L8 in cucumber against seedling damping-off in cucumber

To test the ability of the PGPR strain L8 to induce systemic resistance in cucumber against seedling damping-off caused by the fungal pathogen *P. aphanidermatum*, cucumber seedlings were root-drenched with L8 suspension using a root-split system. As shown on Table 1, L8 treatment effectively protected the cucumber from seedling damping-off as judged by the markedly improved seedling survival rates at 14 and 28 days after inoculation. The relative control efficacy at 28 days was 75.78%.

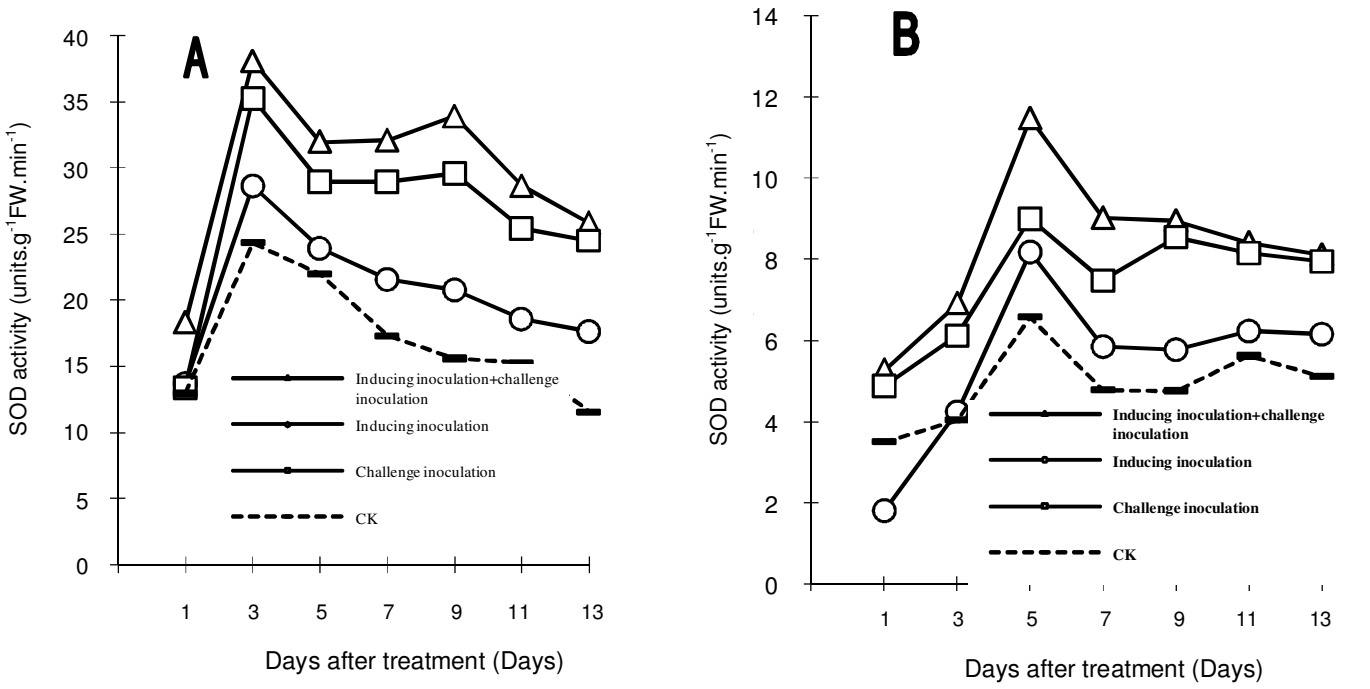

Figure 1. Effects of *B. megaterium* L8 treatment and *P. aphanidermatum* inoculation on superoxide dismutase (SOD) activity

Effects of *B. megaterium* L8 treatment on the levels of SOD activity in the roots and leaves of cucumber

To examine the effect of L8 root colonization on the levels of SOD activity in cucumber, we monitored the enzymatic activity profile of SOD at various time points after inoculation. The dramatic induction of SOD activity in the root (Figure 1a) was observed during the first 3 days after treatment and the levels of SOD activity peaked at 3 days and then declined gradually. The levels of SOD activity were significantly higher in all the treatments compared with the control. The extent of SOD induction in the roots was in the order of: *P. aphanidermatum* challenging inoculation plus L8 root-drenching>*P. aphanidermatum* inoculation only>L8 root-drenching only. The induction pattern of SOD activities in the leaves (Figure 1b) during the same time course, to a great extent, matched that in the roots. However, the magnitude of SOD induction in the leaves (range: 3.5 to 9.1 U) was much smaller than that observed in the treated roots (range: 11.58 to 38.0 U). Furthermore, the maximum levels of SOD activity in the leaves occurred at 5 day after treatment. The shared induction pattern of SOD activities in the time course in the treated roots and untreated leaves suggests that L8 colonization of cucumber roots systematically induced SOD activity in cucumber. The enhanced expression patterns of SOD suggest that *B. megaterium* L8 might accounted for its ability to provide effective protection for cucumber from seedling damping-off caused by the soil-borne fungal pathogen *P. aphanidermatum*. The induced ability of resistance to *P. aphanidermatum* was systemic.

Effects of *B. megaterium* L8 treatment on the levels of POD activity in the roots and leaves of cucumber

The rapid induction of POD activity in the roots (Figure 2a) was observed during the first 3 days after treatments and the levels of POD activity peaked at 3 days and then declined gradually. The levels of POD activity were significantly higher in all the treatments compared with the control. POD activities in the roots treated with *P. aphanidermatum* plus L8 root-drenching was significantly higher than either *P. aphanidermatum* inoculation alone or L8 root-drenching alone. The induction pattern of POD activities in the seedling leaves (Figure 2b) in the time course paralleled that in the roots which is consistent with our hypothesis that induction of POD activity is associated with induced resistance in cucumber against seedling damping-off. Moreover, L8 root-drenching markedly enhanced POD activities in the untreated leaves, suggesting that L8 root colonization could systemically induce POD activity in cucumber.

Effects of *B. megaterium* L8 treatment on the levels of CAT activity in the roots and leaves of cucumber

The levels of CAT activity in the roots experienced (Figure 3a) a rapid increase after treatments. CAT activities in all the treatments were significantly higher than that in the control. The levels of CAT activity in the roots treated with *P. aphanidermatum* challenge inoculation plus L8 root-drenching induction were significantly

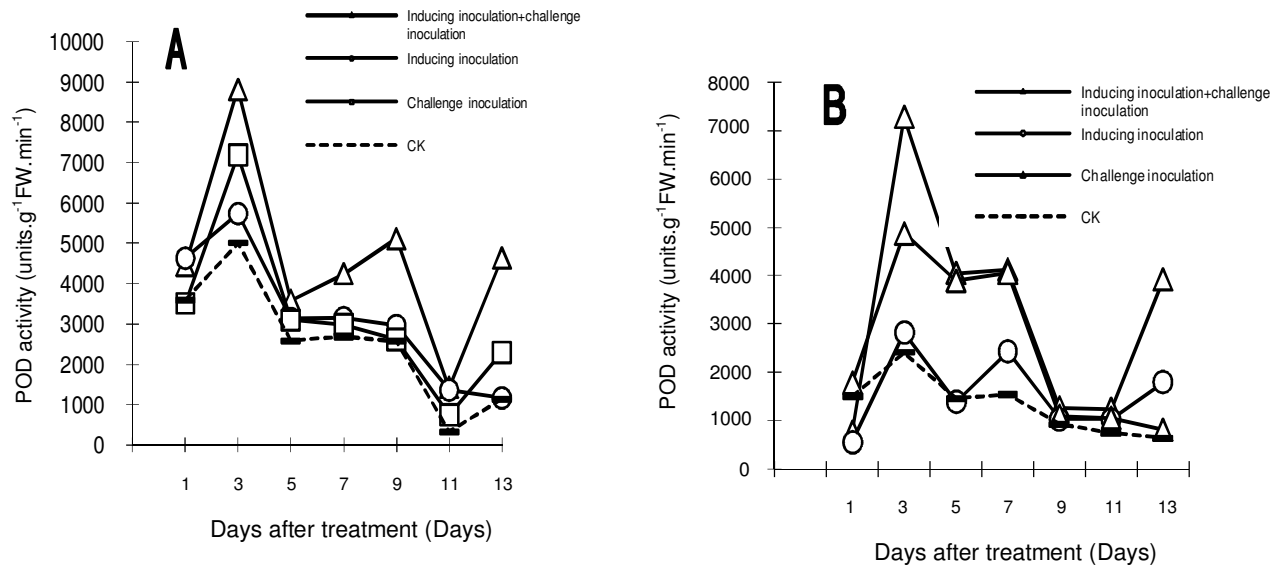

Figure 2. Effects of *B. megaterium* L8 treatment and *P. aphanidermatum* inoculation on peroxidase (POD) activity in the roots and leaves of cucumber. A, In roots; B, in leaves.

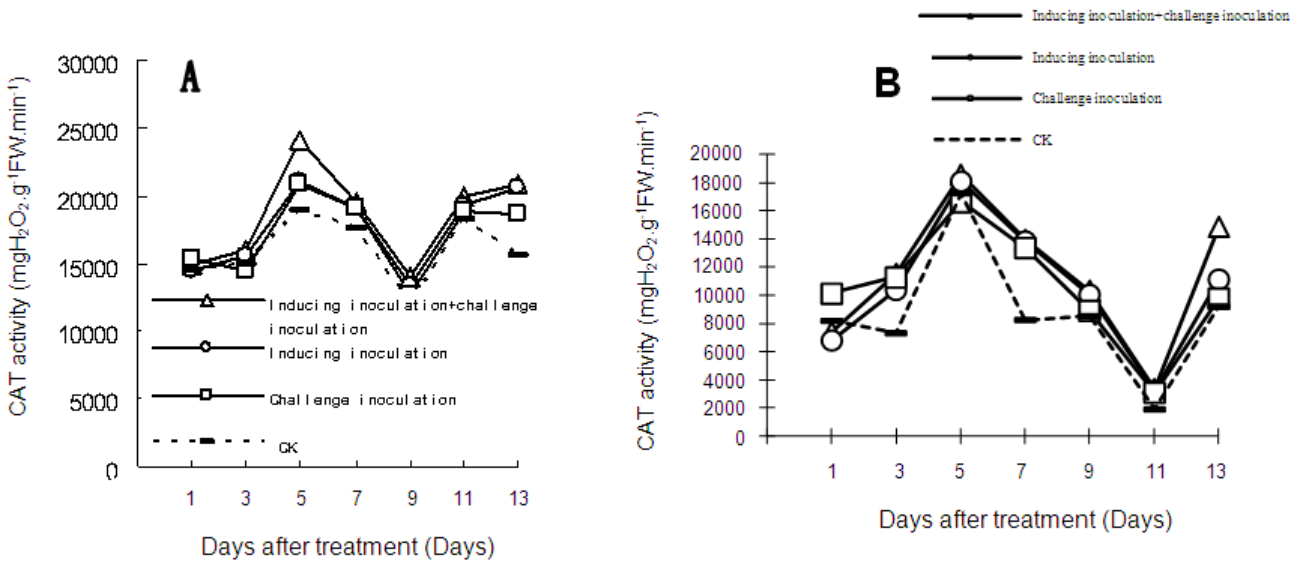

Figure 3. Effects of *B. megaterium* L8 treatment and *P. aphanidermatum* inoculation on catalase (CAT) activity in the roots and leaves of cucumber. A, In roots; B, in leaves.

higher than either *P. aphanidermatum* inoculation alone or L8 root-drenching alone. The induction pattern of CAT activities in the seedling leaves (Figure 3b), for the duration of the experiment, paralleled that in the roots which is consistent with the notion that induction of CAT activity is associated with induced resis-tance in cucumber against seedling damping-off. Moreover, *B. megaterium* L8 root-drenching markedly enhanced CAT activities in the untreated leaves, suggesting that L8 root colonization could systemically induce CAT activity in cucumber.

Effects of *B. megaterium* L8 treatment on the levels of PPO activity in the roots and leaves of cucumber

The levels of PPO activity in the roots exhibited a wave-like induction pattern (Figure 4a) after treatments and manifested two peaks at 5 and 11 days, respectively. PPO activities in all the treatments were significantly higher than that in the control. The extent of PPO induction in the roots was in the order of: *P. aphanidermatum* challenging inoculation + L8 root-drenching > *P.*

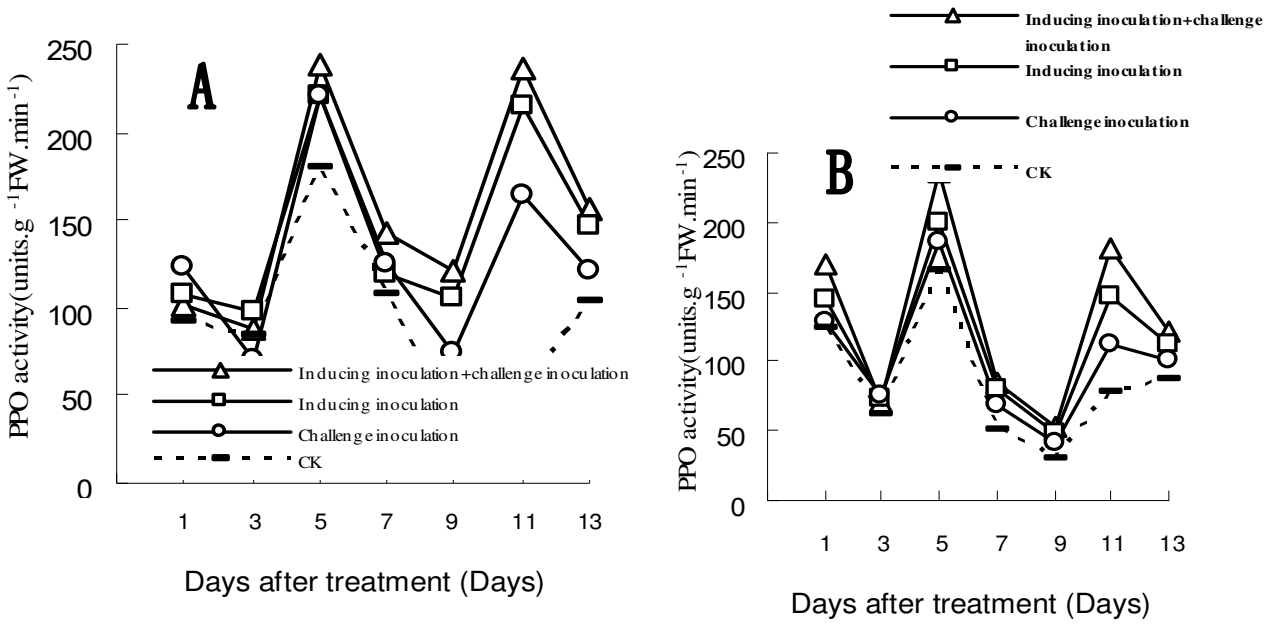

Figure 4. Effects of *B. megaterium* L8 treatment and *P. aphanidermatum* inoculation on polyphenoloxidase (PPO) activity in the roots and leaves of cucumber. A, In roots; B, in leaves.

aphanidermatum inoculation only>L8 root-drenching only. The induction pattern of PPO activities in the seedling leaves (Figure 4b), in the time course, paralleled that in the roots and is consistent with our hypothesis that induction of PPO activity is related to induced resistance in cucumber against seedling damping-off. Moreover, L8 root-drenching markedly enhanced PPO activities in the untreated leaves, suggesting that L8 root colonization could systemically induce PPO activity in cucumber.

Effects of *B. megaterium* L8 treatment on the levels of PAL activity in the roots and leaves of cucumber

The levels of PAL activity in the roots displayed a wavelike induction pattern (Figure 5a) after treatments and showed two peaks at 5 and 11 days, respectively. PAL activities in all the treatments were significantly higher than that in the control. The degree of PAL induction in the roots was in the order of: *P. aphanidermatum* challenging inoculation+L8 root-drenching>*P. aphanidermatum* inoculation only>L8 root-drenching only. The induction pattern of PAL activities in the seedling leaves (Figure 5b), for the duration of the experiment, paralleled that in the roots and was consistent with our hypothesis that induction of PAL expression is associated with induced resistance in cucumber against seedling damping-off. Moreover, a similar induction pattern of PAL activities was observed in the untreated leaves and treated roots, suggesting that L8 root colonization could systemically induce PAL activity in cucumber.

DISCUSSION

Systemically induced enzymatic activity was determined by the split root system. Split cucumber roots were treated with the bacterial strain *B. megaterium* L8 or *P. aphanidermatum* on one side and pathogen inoculation or not on the distant side. The results indicated that L8 treatment effectively protected cucumber from seedling damping-off as judged by the markedly improved seedling survival rates at 14 and 28 days after inoculation. The relative control efficacy at the 28th day was 75.78%. The fact that L8 treatment conferred effective protection against damping-off of cucumber under the experimental condition in which the PGPR L8 was spatially separated from the challenging fungal pathogen *P. aphanidermatum* (using the root-split system) clearly suggests that systemic induction of resistance in cucumber is the primary mechanism underlying the protective effects of L8 in this experimental setting.

When plants are invaded by micro-organisms or damaged by mechanical injuries, major physiological changes are induced and plant defense enzymes are generally activated. In spite of major advances in our understanding of the plant defense response, little information is available on PGPR-mediated induced enzymes (Chen et al., 2000). In this study, two of the PGPR strains, *Pseudomonas* strains 13 and 63-28, significantly induced plant defense enzymes both locally and systemically.

These two strains have been shown to suppress cucumber root disease caused by *P. aphanidermatum* (Chen et al., 1998). In our studies, when cucumber roots were infected with the pathogen, the tested enzyme

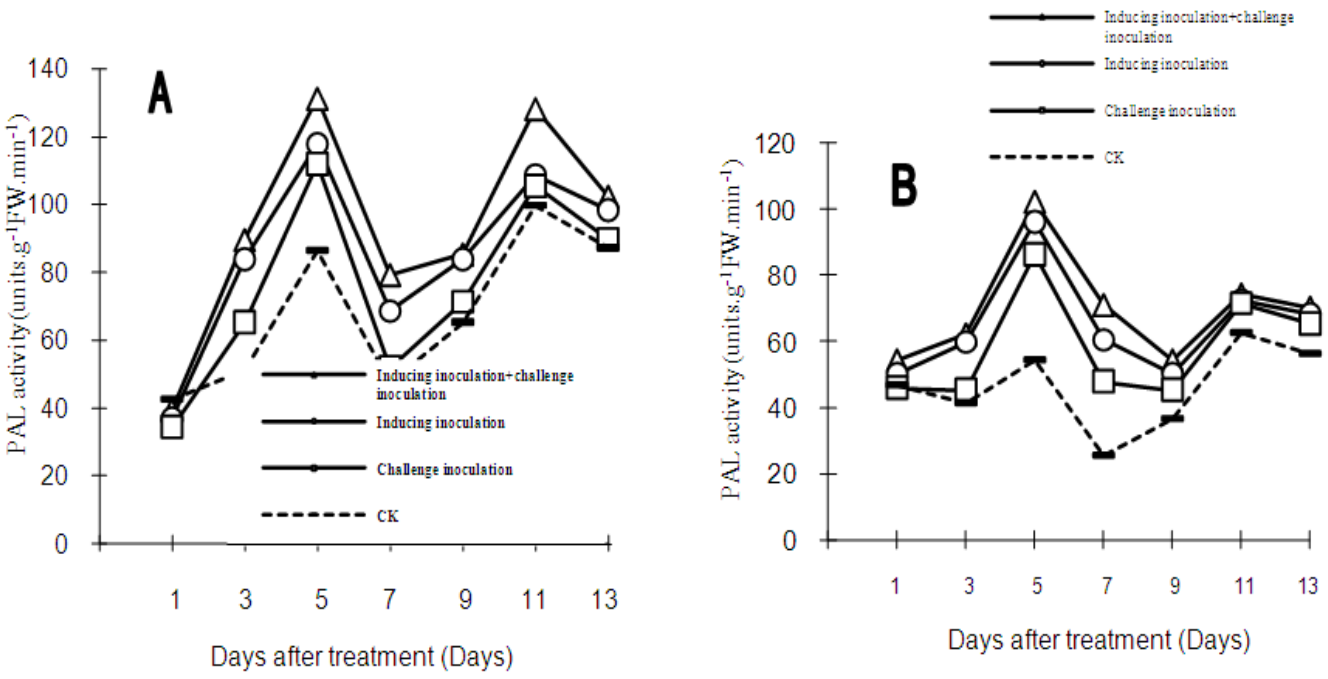

Figure 5. Effects of *B. megaterium* L8 treatment and *P. aphanidermatum* inoculation on phenylalanine ammonia-lyase (PAL) activity in the roots and leaves of cucumber. A, In roots; B, in leaves.

activities increased over time as the disease developed. When PGPR or the root pathogen penetrated the cucumber root system, the enzyme activities increased systemically. In general, the pathogen stimulated more enzyme accumulation than PGPR alone in the cucumber roots. However, the mechanisms underlying the induction process may be different.

Induced disease resistance is an active plant defense process that depends on physical or chemical barriers in the host and is activated by biotic or abiotic inducing agents (Thomashow, 1996). Previous studies reported that different inducers could enhance enzyme activities in cucumber and tobacco plants. Cucumber powdery mildew pathogen (*Sphaerotheca fuliginea*), *Pseudomonas syringae* pv. *pisi*, silica and salicylic acid stimulated enzyme activities of chitinase, β-1, 3-glucanase, PO, PPO and PAL in leaf tissues of cucumber or tobacco plants 2 to 3 days after induction (Schneider, 1994). These enzymes were also activated by treatments with different plant extracts (Mosch et al., 1993) and ethylene (Ke and Saltveit, 1989).

SOD, POD and CAT are antioxidant enzymes involved in the removal of reactive oxygen species; of them, SOD functions as the first line of plant defense against oxidative damage under various stress conditions (Alscher et al., 2002). POD, part of the PR-9 family, is of the lignin-forming type of plant defense response (Van Loon, 1997). Plant POD has been reported to catalyse the last steps in the biosynthesis of lignin and hydrogen peroxide (Bruce and West, 1989). In our studies, three enzymes increased in the plants treated with *B. megaterium* L8 or challenged with *P. aphanidermatum*; induced to higher levels in all the plants than the control. Moreover, *B. megaterium* L8 root-drenching markedly enhanced three enzymes activities in the untreated leaves, suggesting that L8 root colonization could systemically induce three enzymes activities in cucumber.

PAL, one of the key enzymes in the phenylpropanoid pathway and the flavonoid pathway, was increased in both incompatible and compatible interactions between plants and pathogens. Recent work also demonstrated the existence of phenolic phytoalexins in cucumbers, which may be produced through a PAL pathway (O'Neill et al., 1994). In our study, PAL was induced in roots inoculated with *P. aphanidermatum*. Plants treated with *B. megaterium* L8 had higher levels of PAL than the control when these plants were inoculated with the pathogen; the levels were higher by comparison with other treatments or control. The induction of PAL by L8 may have resulted in the activation of defenses. Moreover, *B. megaterium* L8 root-drenching markedly enhanced PAL enzyme activity in the untreated leaves, suggesting that the induced ability of resistance to *P. aphanidermatum* by L8 was systemic.

PPO usually accumulates upon wounding in plants. PPO transcript levels systemically increased in young leaves of tomato when mature leaflets were injured (Thipyapong and Steffens, 1997). In our study, similar to other enzymes, PPO in the cucumber roots and leaves was stimulated by PGPR or the pathogen. Moreover, when cucumber roots were treated with *B. megaterium* L8 and inoculated with *P. aphanidermatum*, the levels in

roots and leaves were higher by comparison with other treatments or control.

In conclusion, the enzymes mentioned earlier played an important role in ISR. Enzyme accumulation could be involved not only in plant defense response, but may also be associated with induced resistance by PGPR *B. megaterium* L8 against cucumber root disease caused by *P. aphanidermatum*. It could be speculated that the enhanced expression patterns of these enzymes by *B. megaterium* L8 might account for its ability to provide effective protection for cucumber from seedling damping-off caused by the soil-borne fungal pathogen *P. aphanidermatum*. The induced ability of resistance to *P. aphanidermatum* was systemic.

ACKNOWLEDGMENTS

The study was supported by grants from the National Natural Science Foundation of China (Grant No. 30800878).

REFERENCES

Alscher RG, Erturk N, Heath L (2002). Role of superoroxide dismutase (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 72: 1331-1341.

Avdiushko SA, Ye XS, Kuć J (1993). Detection of several enzymatic activities in leaf prints cucumber plant. Physiol. Mol. Plant Pathol. 42: 441-454.

Bashan Y, Okon Y, Henis Y (1985). Peroxidase, polyphenoloxidase, and phenols in relation to resistance against *Pseudomonas syringae* pv. *tomato* in tomato plants. Can. J. Bot. 65: 366-372.

Beaudoin-Eagan LD, Thorpe TA (1985). Tyrosine andphenylalanine ammonia lyase activities during shoot initiation in tobacco callus cultures. Plant Physiol. 78: 438-441.

Bruce RJ, West CA (1989). Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures caster bean. Plant Physiol. 91: 889-897.

Chen C, Bélanger RR, Benhamou, N, Paulitz TC(1998). Induced systemic resistance (ISR) by *Pseudomonas* spp. impairs pre- and post-infection development of *Pythium aphanidermatum* on cucumber roots. Eur. J. Plant Pathol. 104: 877-886.

Chen C, Bélanger RR, Benhamou N, Paulitz TC (1999). Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots. Eur. J. Plant Pathol. 105: 477-486.

Chen C, Bélanger RR, Benhamou N, Paulitz TC(2000). Defence enzymes Induced in cucumber roots by treatment with plant growth-promoting rhizobacteria(PGPR) and *Pythium aphanidermatum*. Physiol. Mol. Plant, 56: 13-23.

Curtis MD, Rae AL, Rusu AG, Harrison SJ, Manners JM (1997). A peroxidase gene promoter induced by phytopathogens and methyl jasmonate in transgenic plants. Mol. Plant Microbiol. Interact. 10: 326-338.

Goy AP, Felix G, Metraux JP, Meins F Jr(1992). Resistance to disease in the hybrid *Nicotiana glutinosa* X *Nicotiana debneyi* is associated with high constitutive level of β-1,3-glucanse, chitinase, peroxidase and polyphenoloxidase. Physiol. Mol. Plant Pathol. 41:11-21.

Jetiyanon K, Tuzun S, Kloepper JW (1997). Lignification, peroxidase and superoxidase dismutases as early plant defense reactions associated with PGPR-mediated induced systemic resistance. In Plant Growth-Promoting Rhizobacteria: Present Status and Future Prospects. Ogoshi, A, Kobayashi K, Homma Y, Kodama F, Kondo N, Akino S (Eds.). Sapporo, Jpn. pp. 265-268.

Ke D, Saltveit Jr. ME (1989). Developmental control of russet spotting, phenolic enzymes, and IAA oxidase in cultivars of iceberg lettuce. J. Am. Soc. Hort. Sci., 114: 472-477.

Kloepper JW, Tuzun S, Kuc J (1992). Proposed definitions related to induced disease resistance. Biocont. Sci. Technol. 2: 349-351

Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G (1994). Induction of systemic resistance of tobacco totobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: Influence of the *gac*A gene and of pyoverdine production. J. Phytopathol. 84: 139-146.

Moerschbacher BM, Noll UM, Flott BE (1998). Lignin biosynthetic enzymes in stem rust infected, resistance and susceptible near-isogenic wheat lines. Ann. Mol. Pathol. 33(1): 33-46.

Mosch J, Mende A, Zeller W, Rieck M, Ullrich W (1993). Plant extracts with a resistance induction effect against fire blight(*Erwinia amylo.ora*). Acta Hortic. 338: 389-395.

Mozzetti C, Ferraris L, Tamietti G (1995). Variation in enzyme activities in leaves and cell suspension as markers of incompatibility in different *Phytophthora*-pepper interactions. Physiol. Mol. Plant Pathol. 46: 95-107.

O'Neill NR, Saunders JA (1994). Compatible and incompatible response in alfalfa cotyledons to races 1 and 2 of *Colletotrichum trifolii*. J. Phytopathol. 83: 284-287.

Podile AR, Laxmi VDV (1998). Seed bacterization with *Bacillus subtilis* AF1 increases phenylalanine mmonia-lyase and reduces the incidence of Fusarium wilt in pigeonpea. J. Phytopathol. 146: 255-259.

Press CM, Wilson M, Tuzun S (1997). Salicylic acid produced by Serratia marcescens 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. Mol. Plant Microbe Interact. 10: 761-768.

Rajappan K, Vidhyasekaran P, Raguchander T (1995). Elicitation of enzyme activity by nickel nitrate in suspension cultured rice. Plant Dis. Res. 10: 142-145.

Schneider S(1994). Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. Physiol. Mol. Plant Pathol. 45: 291-304.

Thipyapong P, Steffens JC (1997). Tomato polyphenol oxidase. Differential response of the polyphenol oxidase Fpromoter to injuries and wound signals. Plant Physiol. 115: 409-418.

Thomashow SL (1996). Biological control of plant root pathogens. Curr. Opin. Biotechnol. 7: 343-347.

Van Der Plaats-Niterink AJ(1981). Monograph of the Genus *Pythium*. Study Mycol. 21: 1-142.

Van Loon LC(1997). Induced resistance in plants and the role of pathogenesis-related proteins. Eur. J. Plant Pathol. 103: 753-765.

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998). Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36: 453-83.

Van Peer R, Niemann GJ, and Schippers B (1991). Induced resistance and phytoalexin accumation in biological control of Fasurium wilt of carnation by *Pseudomonas* sp. Strain WCS417r. J. Phytopathol. 81: 728-734.

Wei G, Kloepper JW, Tuzun S (1991). Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. J. Phytopathol. 81: 1508-1512.

Zhou Q (1995). Guide for Plant Physiological and Biochemical Experimentation. China Agric. Press, Beijing, p. 86.

Zhou T, Paulitz TC(1994). Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. on cucumber. J. Phytopathol. 142: 51-63.

Zhou YH, Yu JQ, Qian QQ (2003). Effects of chilling and low light on cucumber seedling growth and their antioxidative enzyme activities. Chin. J. Appl. Ecol. 14(6): 921-924.

Zhu GL, Zhong HW, Zhang AQ(1990). Plant Physiological Experimentation. Peking Univ. Press, Beijing, pp. 37-39.