Journal of Microbiology (2014) Vol. 52, No. 10, pp. 879–883
DOI 10.1007/s12275-014-4399-z

Microscopical Observation of Inhibition-behaviors against *Diaporthe citri* by Pre-treated with *Pseudomonas putida* Strain THJ609-3 on the Leaves of Citrus Plants

Yun Jung Ko[1], Ju Sung Kim[1], Ki Deok Kim[2], and Yong Chull Jeun[1]*

[1]*Major of Plant Resources and Environment, College of Applied Life Sciences, The Research Institute for Subtropical Agriculture and Biotechnology, Jeju National University, Jeju 690-756, Republic of Korea*
[2]*Division of Biotechnology, Korea University, Seoul 136-713, Republic of Korea*

(Received Jul 7, 2014 / Revised Aug 13, 2014 / Accepted Aug 18, 2014)

Citrus melanose is one of the most important diseases in orchards cultivating citrus in the world. Although the disease does not cause yield loss, the profitability of the infected fruits is often reduced in the fresh-market, resulting in economic loss. In this study, disease reduction was proven by pre-treatment with *Pseudomonas putida* strain THJ609-3. In order to illustrate mechanism of the disease reduction by the bacterial strain, the infection behaviors of *Diaporthe citri* and necrosis deposit of plant tissue were observed using a fluorescence microscope. On the leaves pre-treated with the strain THJ609-3, germination rates of *D. citri* conidia were significantly decreased compared to those of the untreated control. Scanning electron microscopical observations showed that bacterial cells were attached to the surface of fungal hyphae. Furthermore, morphological change of germ tubes of the conidia was detected. These results suggest that the disease reduction may be caused by the direct antifungal activity of the bacterial strain on the leaf surfaces.

Keywords: bio-fungicide, biological control, melanose, Satsuma mandarin

Introduction

Melanose caused by *Diaporthe citri* F.A. Wolf (asexual phase: *Phomopsis citri* H. Fawc) is one of the most important diseases in citrus growing areas of the world (Mondal *et al.*, 2007). Although it does not cause yield loss, it reduces profitability of the fresh-market. In Florida, USA, serious loss by melanose has been reported in fresh-market of grapefruit (Timmer *et al.*, 1998). In Korea melanose usually occurs, from June to August in the orchard cultivating Satsuma mandarin in Jeju, which is the most popular citrus cultivar in the island (Kwon *et al.*, 2003). The infection rate of the fruits by the pathogen could reach to 95% in adult citrus trees when there were no applications of control measures (Hur and Park, 2005).

The inoculum is produced in pycnidia on old dead twigs, not on any of the living tissues (Whiteside, 2000). The pathogen invades on leaves, twigs, and fruits at extended wetting periods and high temperatures (Agostini *et al.*, 2003). Although ascospores serve to disseminate the disease widely, it seems not to play a critical role as a major inoculum (Mondal *et al.*, 2004).

Most of the protection strategies against melanose in Satsuma mandarin depend on chemical treatment such as dithiocarbamate (Mancozeb®), whose amount of usage reaches 65% of the total amount of chemical usage in the citrus in Jeju (Kim *et al.*, 2005). In some orchards, copper products have been applied for reducing melanose, which often results in frequent occurrence of side effects such as phytotoxicity (Hyun *et al.*, 2005). Therefore, alternative control measures for this disease are highly demanded due to the occurrence of fungicide resistant pathogens or environmental problems caused by indiscreet application of fungicide. Moreover, consumer interest in the safety of fresh agricultural products has increased.

Biological control using effective microorganisms has been known as an environmentally friendly method in plant protection (Martins *et al.*, 2013; Nawrocka and Malolepsza, 2013). One biological control may rely on using plants expressing induced systemic resistance (ISR), which is mediated by plant growth promoting rhizobacteria (PGPR) (Sang *et al.*, 2014). In the last decade, disease control by ISR has been intensively studied in many anneal crop plants because it circumvents all the problems associated with chemical control (Zehnder *et al.*, 2001).

However, only a few strudies have reported biological control in citrus trees. In our previous study two effective bacterial strains against melanose on citrus leaves were selected (Ko *et al.*, 2012). In this study disease inhibition on the citrus leaves by *Pseudomonas putida* strain THJ609-3 was investigated using a fluorescens microscopy. Also, the bacterial strain attached on the melanose pathogen was observed via the use of a scanning electron microscopy.

Materials and Methods

Plant

The seedlings of Satsuma mandarin (Sunmyeong®, Nongwoo Bio, Korea) which was grafted with the root of trifoliate or-

*For correspondence. E-mail: ycjeun@jejunu.ac.kr

ange, were planted in plastic pots (Ø 25 cm, 40 cm high) filled with commercial soil (Tuksimi®, Nongwoo green Tec., Korea) sterilized at 80°C for 4 h. The seedlings were grown in a greenhouse maintained at 60% relative humidity (RH), 25±1°C, with 4,000 lux illumination during day time and 20±1°C at night. The seedlings were watered every 3 days and fertilized once a week with 30 ml of a complex fertilizer Choroc Nala® (N-P-K, 30-10-10, Bokyung Nongsan, Korea) as recommended for commercial usage.

Sections of the tops of the branches, containing 5–6 leaves were detached from the seedling with a sterile blade and were put in a flask containing distilled water. These branches were used for fungal inoculation and for the fluorescence and electron microscopical observation.

Treatment of plants with bacterial isolate and fungicide

The reduction of disease severity by bacterial strain *Pseudomonas putida* THJ609-3 against melanose was screened using the method described in Ko *et al.* (2012). The bacterial strain was freshly grown on trytic soy agar (TSA) medium at 28°C for 48 h. Suspension of the bacterial strain was prepared at the concentration of 1.0×10^6 CFU/ml, and each flask of a citrus branch was sprayed with the bacterial suspension. In order to evaluate the efficacy of the bacterial strain, solution of a commercial fungicide Dithianon® was sprayed at the concentration of 1 g/L as a control. The treated citrus branches were sustained at room temperature for 6 h until fungal inoculation.

Pathogen

Citrus melanose pathogen *Diaporthe citri* was grown on potato dextrose agar medium at 25°C for 14 days. To induce conidia the plates were incubated under fluorescence lamp at the same temperature for 7 days. After conidial formation, 10 ml distilled water was poured into each culture plate and harvested conidia with a loop. The conidial suspension was filtered through two layers of miracloth, and the concentration of conidia was adjusted to 1.0×10^6 conidia/ml for inoculation with fungal pathogen.

Fungal inoculation and assessment of citrus melanose disease

The conidial suspension of *D. citri* containing 0.01% Tween 20 was sprayed onto upper and lower surfaces of the leaves of untreated and bacterial strain pre-treated citrus branch. The inoculated plants were kept in a dew chamber maintained at 100% RH in the dark at 28 ± 1°C for 48 h and then transferred to a growth chamber maintained at 60% RH and at 23/28°C (night and day, respectively) until symptoms of melanose appeared. The level of disease severity was determined at 7 days after the fungal inoculation by counting the lesion number per leaf. Three independent experiments were carried out with three replications containing 5 citrus branches each.

Fluorescence microscopic observation of the infection structure on the leaf surface

Microscopic observations of infection behavior of *D. citri* and necrosis deposits of plant tissue on the surface of infected citrus leaves were performed at 3 days after fungal inoculation. Staining of the leaf tissues was carried out as described by Jeun *et al.* (2000). The infected part of leaf was cut out with a razor blade in size of 1×3 mm^2, and fixed in 2% (v/v) glutaraldehyde in 0.05 M phosphate buffer (pH 7.4) for 2 h. After washing with the phosphate buffer three times for 10 min each, the sections were stained with 0.5% (w/v) aniline blue for 20 min to detect callose formation at the penetration sites. After washing with the buffer, the leaf disks were stained with 0.02% Uvitex 2B (w/v) (Diethanol) for 20 min, which is a fluorochrome for 1,3-β-glucans. After washing, the leaf disks were mounted on glass slides with 50% glycerin.

The infection structures of *D. citri* were observed using a fluorescence microscope (Olympus, Japan) equipped with a 'U' exciter cube-filter and an ultraviolet epifluorescence filter set (BP 400-440, FT 460, LP 470). Total numbers of conidia and germinated conidia were counted and necrosis depositions were determined on the leaves pre-treated with rhizobacterial isolates and non-treated. Germination rate (%) was calculated as (number of germinated conidia) / (total number of conidia) × 100. And necrosis rate (%) was calculated as (number of necrosis deposits) / (total number of conidia) × 100. Five leaves were randomly detached from five plants for each experiment and three independent experiments in total were performed.

Scanning electron microscopic observation of the fungal structure on the leaves

Pre-treated with the bacterial strain and non-treated leaf surfaces of citrus leaves were observed at 3 days after inoculation with *D. citri* using a scanning electron microscope (SEM 435 VP, Leo 40 Electron Microscopy Ltd., UK).

The inoculated leaves were cut into 0.4×0.6 mm^2 using a sterile blade. Fixation, dehydration and embedding of the roots were performed according to Hayat (1989). The leaf samples were fixed in 2% (v/v) glutaraldehyde in 0.05 M phosphate buffer (pH 7.4) for 2 h. After washing with the same phosphate buffer for 10 min three times each, post fixation was performed in 2% (v/v) osmium tetroxide in phosphate buffer for 2 h at room temperature. After washing three times, the samples were dehydrated through an alcohol series (25, 50, 70, 90, and 100% two times for 30 min each). The samples were gently dried using a critical point drier (CPD 030, BAL-Tec). Samples were mounted on metallic stubs, gold-coated (~100 Å) with a sputter coater (Polaron Sputter Coat System, Model 5001, England) and viewed under SEM 435 VP at 20 kV. The numbers of bacteria attached fungal conidia and germ tubes were randomly selected and counted.

Statistical analyses

The evaluation of disease severity of citrus branches after inoculation with *D. citri* was carried out as 3 separate experiments at different (n=9).

Under the fluorescence microscopic observation the number of necrosis deposits on the infected citrus leaves and the number of fungal conidia and germ tubes were statisti-

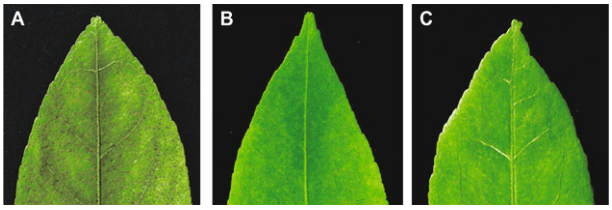

Fig. 1. Leaves of citrus plants pre-treated with H_2O (A), pre-treated with bacterial strain *P. putida* THJ609-3 (B) and commercial fungicide Dithianon® (C). The concentration of the bacterial suspension and the solution of fungicide were 1.0×10^6 CFU/ml and 1 g/L, respectively. The photographs were taken at 7 days after inoculation with suspension of melanose pathogen *D. citri* at the concentration of 1.0×10^6 conidia/ml. The experiments were carried out with three independent replications.

cally analyzed using Duncan's multiple range tests (DMRT). Statistical analysis of the experimental data was conducted using the Statistical Analysis System (SAS institute, version 9.0).

Under the scanning electron microscopic observation the number of bacterial cells attached to the fungal conidia and hyphae from the bacterial strain treated and untreated citrus leaves was compared using a paired t-test. Significance levels of P = 0.001 were used for the statistical test.

Results

Suppression of disease severity by pre-treatment with bacterial strain

On the non-treated plants, the typical melanose symptom was observed at 7 days after inoculation with *D. citri*. Many lesions were found on the untreated control leaves (Fig. 1A). In contrast to control leaves, the lesion numbers were apparently decreased at the same time on the leaves pre-treated with *P. putida* THJ609-3 (Fig. 1B). These results showed that disease severity of melanose may be suppressed by treatment with the bacterial strain. On the leaves pre-treated with the commercial fungicide no lesions were observed at 7 days after the fungal inoculation (Fig. 1C) indicating strong suppression of disease development by the fungicide.

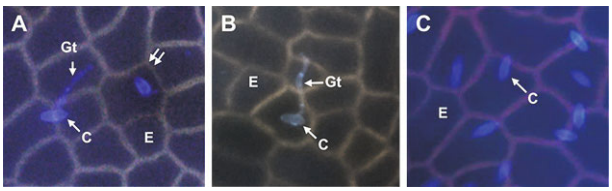

Fig. 2. Fluorescent microscopic observations of infection behavior of melanose pathogen *D. citri* on the leaves of citrus plants pre-treated with H_2O (A), bacterial strain *P. putida* THJ609-3 (B) and commercial fungicide Dithianon® (C). The concentration of the bacterial suspension and the solution of fungicide were 1.0×10^6 CFU/ml and 1 g/L, respectively. The images represented the citrus leaves at 3 days after inoculation with the suspension of *D. citri* at the concentration of 1.0×10^6 conidia/ml. The experiments were carried out with three independent replications. Abb.: C, conidium; E, epidermal cell; Gt, germ tube (bars = 20 μm).

Fluorescent microscopic observation of the infection structure on the leaf surface

The infection behavior of *D. citri* was observed on the leaf surfaces of the citrus leaves both pre-treated with the bacterial strain *P. putida* THJ609-3 and non-treated. On the leaves of untreated leaves conidia and germ tube, which were stained with Uvitex 2B, could be observed (Fig. 2A). Also, necrosis deposits of epidermal cell were found at the site of conidia located (Fig. 2A, double arrows). About 20% of epidermal cells was necroses at the site of conidia located (Fig. 3). Lots of conidia were counted on the leaf surfaces (Fig. 3) of which 20–40% were germinated at 3 days

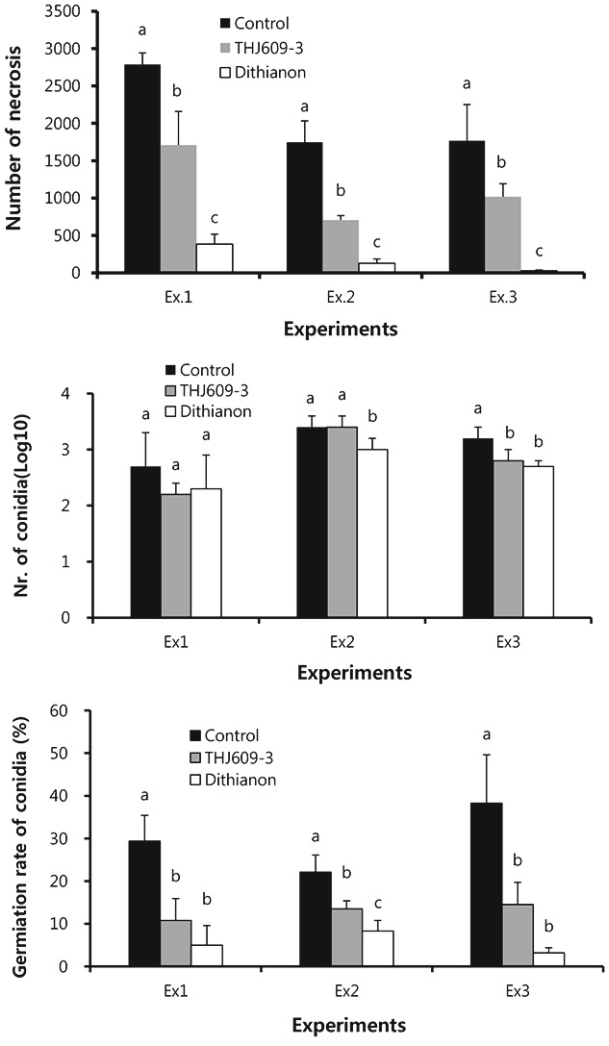

Fig. 3. Number of necrosis, number of conidia and germination rate of conidia on the leaves of citrus plants pre-treated with H_2O, bacterial strain *P. putida* THJ609-3 and commercial fungicide Dithianon®. The concentration of the bacterial suspension and the solution of fungicide were 1.0×10^6 CFU/ml and 1 g/L, respectively. The necrosis deposits, total conidia on the leaf disk and the germinated conidia were counted at 3 days after inoculation with the suspension of *D. citri* at the concentration of 1.0×10^6 conidia/ml. Germination rate was calculated as (number of germinated conidia) / (total number of conidia) × 100 (%). Different letters indicate significant differences (P < 0.05) according to Duncan's multiple range test.

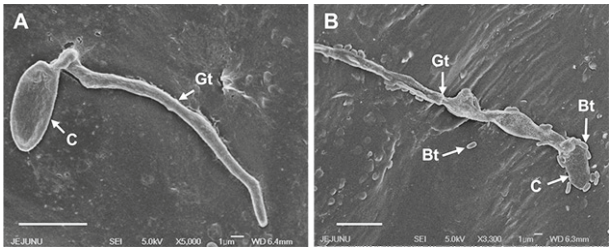

Fig. 4. Scanning electron microscopical observations of melanose pathogen *D. citri* on the leaves of citrus plants pre-treated with H_2O (A) and bacterial strain *P. putida* THJ609-3 (B). The concentration of the bacterial suspension was 1.0×10^6 CFU/ml. The images represented the citrus leaves at 3 days after inoculation with the suspension of *D. citri* at the concentration of 1.0×10^6 conidia/ml. Abb.: C, conidium; Bt, bacterial cell; Gt, germ tube (bars = 5 μm).

after fungal inoculation (Fig. 3). The inoculated leaves were covered with the fungal hyphae two days later (date not shown).

On the leaves pre-treated with *P. putida* THJ609-3, morphological change of the fungal hyphae could not be observed compared to those of untreated control leaves (Fig. 2B). However, the necrosis rate was decreased in all experiments compared to the untreated control leaves (Fig. 3) indicating that the bacterial strain play a role in reduction of the necrosis deposits. The number of conidia was not distinctly decreased (Fig. 3), which indicates that the reduction of the number of conidia on the leaf surface could not be the main factor for suppression of disease severity. Remarkably, germination rate of the conidia was significantly decreased in all experiments (Fig. 3), supporting that the pre-treatment of the bacterial strain may suppress the germination of the fungal conidia.

On the Dithianon® pre-treated leaves the germ tubes were rarely found (Fig. 2C) and the germination rate was apparently decreased compared to those of untreated control leaves (Fig. 3). However, the number of conidia observed on the leaf surface was not apparently decreased (Fig. 3). Consequentially, necrosis of the epidermal cells was very rarely found in all experiments, less than not only untreated control but also the bacterial strain pre-treated leaves (Fig. 3).

Scanning electron microscopic observation of the fungal structure on the leaves

In order to illustrate the role of bacterial strain on disease suppression, the leaves pre-treated with bacterial strain were observed under a scanning electron microscope after inoculation with *D. citri*. On the untreated leaves the conidia was germinated and grown on the leaf surface. There were no bacterial cells surrounding the conidia and term tube (Fig. 4A).

On the leaves pre-treated with *P. putida* THJ609-3 some bacterial cells, which were probably cells of *P. putida* THJ609-3, were attached to the surface of not only the fungal conidia but also the germ tube (Fig. 4B and Table 1). Some germ tubes were morphologically changed, indicating the fungal germ tube reacts to the bacterial cells and the direct antifungal activity of the bacterial cells.

Table 1. Numbers of bacterial cell attached on the fungal surface on the leaves of citrus plants pre-treated with bacterial strain and untreated after inoculation with *D. citri*

Treatment[a]	Number of bacterial cells	*t*-test
P. putida THJ609-3	3.6 ± 2.3[b]	**
Untreated	0.4 ± 0.6	

[a] The concentration of bacterial strains were 1.0×10^6 CFU/ml.
[b] Means ± standard deviation from 10 randomly selected SEM images.
** Significant difference between the bacterial strain treated and untreated plants at the 1% probability level by *t*-test

Discussion

Application with effective bacteria is one of the strategies for plant protection by which the usage of chemical fungicides can be reduced in agriculture (Nawrocka and Malolepsza, 2013). The efficacies of PGPR for reducing disease severity have been reported in many interactions between anneal plant and plant pathogen (Nawrocka and Malolepsza, 2013). In comparison, in a perennial plant such as citrus plant, only a few studies on ISR were carried out. Furthermore, it was very hard to test whether a certain bacterial strain can promote the growth of a shrub like citrus plant.

In this study pre-treatment with the effective bacterial strain *P. putida* THJ609-3 caused the reduction of the total number of melanose lesions on citrus leaves (Fig. 1). This reduction of melanose lesions may be due to the antifungal activity of the bacteria rather than resistance induction mediated by the bacteria. Anyway, these results can indicate that the bacterial strain have potential efficacy to protect the melanose disease in a perennial citrus plants. Many studies have revealed biological control by the antifungal activity of bacterial strains (Ma *et al.*, 2008; Wei-wei *et al.*, 2008; Sowndhararajan *et al.*, 2012).

Infection behaviors of plant pathogen were often observed on the leaf surface of many plants expressing ISR by PGPR (Jeun *et al.*, 2004; An *et al.*, 2010). Mostly, there were no differences on germination rates or appressorium formation rates of fungal pathogen compared to those of untreated control. For example, in cucumber plants pre-treated with *Serratia marcescens* 90-166 or *Pseudomonas fluorescens* 89B61 the germination rate and appressorium formation rate of the anthracnose pathogen *Colletotrichum orbiculare* were not decreased compared to untreated control plants (Jeun *et al.*, 2004). Also, in tomato plant pre-treated with *B. gladioli* TRH 423-3, *Miamiensis avidus* TRH427-2, and *Acinetobacter quenomosp* KRJ 502-1 no differences were found in infection behavior of *Phytophthora infestans* (An *et al.*, 2010).

However, the germination rate of melanose pathogen on the leaves pre-treated with *P. putida* THJ609-3 was significantly decreased in all replicated experiments (Fig. 3). Also, the necrosis rate on the bacteria pre-treated leaves was reduced (Fig. 3). These results indicate that, unlike ISR expressing plants, the suppression of the fungal germination by the bacterial pre-treatment may cause the reduction of the necrosis rate i.e. disease severity. Similar results have been shown in other studies. In canola, a bacterial strain *Bacillus subtilis* QST713 could decrease the germination and viability of *Plasmodiophora brassicae* resting spores (Lahlali *et al.*, 2011).

In contrast, the number of conidia of the melanose pathogen was not decreased on the citrus leaves pre-treated with the bacterial strain (Fig. 3). It seemed that the bacteria could not affect the adhesion of the fungal pathogen on the leaves.

In summary, pre-treatment with the bacterial strain *P. putida* THJ609-3 could reduce melanose disease on citrus leaves. The mechanisms of disease reduction by the bacterial strain *P. putida* THJ609-3 may lay on the suppression of the germination of the fungal conidia and the direct anti-fungal activity to the fungal hyphae. Further studies may be needed to illustrate clearly the mechanisms of disease reduction by the bacterial strain in citrus plants. Probably, other defense response genes such as PR-proteins or growth promoting gene such as plant hormones could be involved (van Loon, 2007). Also, the production of anti-fungal substance phytoalexins may be related the resistance expression mediated by the bacterial strain (Bowles, 1990; Maurhofer *et al.*, 1994).

Acknowledgements

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008264)" Rural Development Administration, Republic of Korea.

References

Agostini, J.P., Bushong, P.M., Bhatia, A., and Timmer, L.W. 2003. Influence of environmental factors on the severity of citrus scab and melanose. *Plant Dis*. **87**, 1102–1106.

An, Y.J., Kang, S.C., Kim, K.D., Hwang, B.K., and Jeun, Y.C. 2010. Enhanced defense responses of tomato plants against late blight pathogen *Phytophthora infestans* by pre-inoculation with rhizobacteria. *Crop Prot*. **29**, 1406–1412.

Bowles, D.J. 1990. Defense-related proteins in higher plants. *Annu. Rev. Biochem*. **59**, 873–907.

Hayat, M.A. 1989. Chemical fixation, pp. 1–78. Principles and techniques of electron microscopy. Biological applications, 3rd ed. The Macmillan Press LTD.

Hur, K.H. and Park, S.K. 2005. Sporulation and dissemination of pycnidiospores of *Diaporthe citri* in yuzu tree (*Citrus junos* Sieb) in Jeonnam Area. *Res. Plant Dis.* **11**, 16–20.

Hyun, J.W., Ko, S.W., Kim, D.H., Han, S.G., Kim, K.S., Kwon, H.M., and Lim, H.C. 2005. Effective usage of copper fungicides for environment-friendly control of citrus diseases. *Res. Plant Dis*. **11**, 115–121.

Jeun, Y.C., Park, K.S., Kim, C.H., Fowler, W.D., and Kloepper, J.W. 2004. Cytological observations of cucumber plants during induced resistance elicited by rhizobacteria. *Biol. Control* **29**, 34–42.

Jeun, Y.C., Sigrist, J., and Buchenauer, H. 2000. Biochemical and cytological studies on mechanisms of systemically induced resistance in tomato plants against *Phytophthora infestans*. *J. Phytopathol.* **148**, 129–140.

Kim, Y.S., Choo, H.Y., Park, C.G., and Lee, D.W. 2005. Analysis of pesticide residues on sweet persimmon harvested from systemized orchards for exporting to USA. *Korean J. Pesticide Sci.* **9**, 166–172.

Ko, Y.J., Kang, S.Y., and Jeun, Y.C. 2012. Suppression of citrus melanose on the leaves treated with rhizobacterial strains after inoculation with *Diaporthecitri*. *Res. Plant Dis.* **18**, 331–337.

Kwon, H.M., Nam, K.W., Kim, K.S., Kim, D.H., Lee, S.C., and Hyun, J.W. 2003. Characterization of the causal fungus of citrus melanose, *Diaphorthe citri* isolated from blighted twigs of citrus in Jeju. *Res. Plant Dis*. **9**, 153–158.

Lahlali, R., Peng, G., McGregor, L., Gossen, B.D., Hwang, S.F., and McDonald, M. 2011. Mechanisms of the biofungicide Serenade (*Bacillus subtilis* QST713) in suppressing clubroot. *Biocontrol Sci. Technol.* **21**, 1351–1362.

Liu, W.W., Mu, W., Zhu, B.Y., DU, Y.C., and Liu, F. 2008. Antagonistic activities of volatiles from four strains of *Bacillus* spp. and *Paenibacillus* spp. against soil-borne plant pathogens. *Agr Sci China*. **7**, 1104–1114.

Ma, Y., Chang, Z.Z., Zhao, J.T., and Zhou, M.G. 2008. Antifungal activity of *Penicillium striatisporum* Pst10 and its biocontrol effect on Phytophthora root rot of chilli pepper. *Biol. Control* **44**, 24–31.

Martins, S.J., Medeiros, F.H.V., Souza, R.M., Resende, M.L.V., and Junior, P.M.R. 2013. Biological control of bacterial wilt of common bean by plant growth-promoting rhizobacteria. *Biol. Control* **66**, 65–71.

Maurhofer, M., Hase, C., Meuwly, P., Mraux, J.P., and Defago, G. 1994. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHAO: influence of the *gacA*-gene and of pyoverdine production. *Phytopathology* **84**, 139–146.

Mondal, S.N., Agostini, J.P., Zhang, L., and Timmer, L.W. 2004. Factors affecting phcnidium production of *Diaporthe citri* on detached citrus twigs. *Plant Dis.* **88**, 379–382.

Mondal, S.N., Vicent, S., Reis, R.F., and Timmer, L.W. 2007. Saprophytic colonization of citrus twigs by *Diaporthe citri* and factors affecting phcnidial production and conidial survival. *Plant Dis*. **91**, 387–392.

Nawrocka, J. and Malolepsza, U. 2013. Diversity in plant systemic resistance induced by *Trichoderma*. *Biol Control*. **67**, 149–156.

Sang, M.K., Kim, E.N., Han, G.D., Kwack, M.S., Jeun, Y.C., and Kim, K.D. 2014. Priming-mediated systemic resistance in cucumber induced by *Pseudomonas azotoformans* GC-B19 and *Paenibacillus elgii* MM-B22 against *Colletotrichum orbiculare*. *Phytopathology*. Article in print.

Sowndrhrarajan, K., Marimuthu, S., and Manian, S. 2012. Biocontrol potential of phylloplane bacterium *Ochrobactrum anthropi* BMO-111 against blister blight disease of tea. *J. Appl. Microbiol.* **114**, 209–218.

Timmer, L.W., Zitko, S.E., and Albrigo, L.G. 1998. Split application of copper fungicides improve control of melanose on grapefruit in Florida. *Plant Dis*. **82**, 983–986.

Van Loon, L.C. 2007. Plant responses to plant growth-promoting rhizobacteria. *Eur J. Plant Pathol.* **119**, 243–254.

Wei-wei, L.I.U., Wei, M.U., Bing-yu, Z.H.U., You-chen, D.U., and Feng, L.I.U. 2008. Antagonistic activities of volatiles from four strains of *Bacillus* spp. and *Paenibacillus* spp. against soil-borne plant pathogens. *Agr. Sci. China* **7**, 1104–1114.

Whiteside, J.O. 2000. Melanose. *In* Timmer, L.W., Garnsey, S.M., and Grahan, J.H. (eds.), Compendium of citrus diseases. 2nd ed., pp. 28–29. The American Phytopathological Society, St. Paul, MN, USA.

Zehnder, G.W., Murphy, J.F., Sikora, E.J., and Kloepper, J.W. 2001. Application of rhizobacteria for induced resistance. *Eur. J. Plant Pathol.* **107**, 39–50.