

Special Issue | Open Access

Determinants of Plant Growth-promoting *Ochrobactrum lupini* KUDC1013 Involved in Induction of Systemic Resistance against *Pectobacterium carotovorum* subsp. *carotovorum* in Tobacco Leaves

Marilyn Sumayo, Mi-Seon Hahm and Sa-Youl Ghim*

School of Life Sciences and Institute for Microorganisms, Kyungpook National University, Daegu 702-701, Korea

(Received on September 13, 2012; Revised on September 21, 2012; Accepted on November 7, 2012)

The plant growth-promoting rhizobacterium *Ochrobactrum lupini* KUDC1013 elicited induced systemic resistance (ISR) in tobacco against soft rot disease caused by *Pectobacterium carotovorum* subsp. *carotovorum*. We investigated of its factors involved in ISR elicitation. To characterize the ISR determinants, KUDC1013 cell suspension, heat-treated cells, supernatant from a culture medium, crude bacterial lipopolysaccharide (LPS) and flagella were tested for their ISR activities. Both LPS and flagella from KUDC1013 were effective in ISR elicitation. Crude cell free supernatant elicited ISR and factors with the highest ISR activity were retained in the *n*-butanol fraction. Analysis of the ISR-active fraction revealed the metabolites, phenylacetic acid (PAA), 1-hexadecene and linoleic acid (LA), as elicitors of ISR. Treatment of tobacco with these compounds significantly decreased the soft rot disease symptoms. This is the first report on the ISR determinants by plant growth-promoting rhizobacteria (PGPR) KUDC1013 and identifying PAA, 1-hexadecene and LA as ISR-related compounds. This study shows that KUDC1013 has a great potential as biological control agent because of its multiple factors involved in induction of systemic resistance against phytopathogens.

Keywords : biological control, induced systemic resistance (ISR), *Ochrobactrum lupini* KUDC1013, plant growth-promoting rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR) are non-pathogenic bacteria colonizing the surface of plant roots with beneficial effects on plant health and growth, provide protection to some unfavorable environmental conditions, suppress phytopathogens and accelerates nutrient availability and assimilation (Babalola, 2010). The use of PGPR is an environment-friendly alternative to control plant diseases commonly caused by deleterious pathogens. Plant diseases

are often controlled using resistant plants and hazardous chemicals. However, resistance does not occur against a wide array of diseases and producing resistant plants takes many years (Lugtenberg and Kamilalova, 2009) while application of chemicals to enhance plant growth or induce resistance is limited because of the negative effects of chemical treatment as well as the difficulty to determine the optimal concentrations to benefit plants (Ryu et al., 2005).

The utilization of PGPR is considered as a favorable approach to augment plant immunity and to control plant diseases. PGPR has diverse mechanisms in controlling diseases including direct antibiosis (Chin et al., 1998; Dunne et al., 1998; Haas et al., 2003), competition of nutrient and niches (Han et al., 2006; Kamilova et al., 2005) and induction of systemic resistance (ISR) (Jetiyanon and Kloepper, 2002; van Peer et al., 1991). ISR is an elevated resistance observed in the whole plant and not only at the site colonized by the rhizobacteria (Kloepper et al., 1992). ISR is phenotypically similar to systemic acquired resistance (SAR) which is evident by direct antibiosis between the inducing bacterium and the challenging pathogen and the maximum level of SAR is expressed when the inducing organism causes necrosis (Cameron et al., 1994), whereas ISR by rhizobacteria typically do not cause any necrotic symptoms on the host plants (van Loon et al., 1998). The utilization of SAR inducing organisms has not been successful under field conditions and generally, the duration of protection is less following induction of a pathogen than that with rhizobacteria-mediated ISR (Wei et al., 1991). Also, PGPR mediated ISR is effective against a wide array of fungal, bacterial and viral plant pathogens (van Loon et al., 1998).

Many individual PGPR components induce ISR such as cell wall components flagellin (Gomez-Gomez and Boller, 2002) and lipopolysaccharides (van Peer and Schippers, 1992; Leeman et al., 1995). Various secondary metabolites produced by some PGPR strains were found to elicit ISR such as 4-aminocarbonyl phenylacetate (Park et al., 2009), N-alkylated benzylamine derivative (Ongena et al., 2005), butyl 2-pyrrolidone-5-carboxylate (Park et al., 2009). Some

*Corresponding author.

Phone) +82-53-950-5374, FAX) +82-53-955-5522

E-mail) ghimsa@knu.ac.kr

PGPR strains release volatile organic compounds (VOCs) involved in ISR activity such as 2-3-butanediol and acetoin (Ryu et al., 2004). These PGPR metabolites have received much attention as part of agricultural methodologies that provide an alternative to synthetic chemicals for pathogen control (Park et al., 2009). Thus, finding new microbial metabolites with ISR activity against plant pathogens is of great interest in agriculture.

Ochrobactrum lupini KUDC1013 elicits ISR in pepper against bacterial spot caused by *Xanthomonas axonopodis* pv. *vesicatoria* and in tobacco against leaf soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* (Ham et al., 2009; Hahm et al., 2012). Recently, KUDC1013 has been found to elicit ISR in pepper against fungal pathogen *Stemphylium lycopersici* causing gray leaf spot disease (unpublished data). This strain has great potential as biological control agent especially in eliciting ISR; however, the determinants of KUDC1013 involved in ISR activity remain unknown. The present study aims to identify the determinants of KUDC1013 involved in ISR elicitation. The role of LPS, flagella and cell free extracts of KUDC1013 to confer ISR activity was investigated.

Materials and Methods

Bacterial strains. PGPR strain *Ochrobactrum lupini* KUDC1013, originally isolated from the rhizosphere of *Solanum nigrum* L. in Dokdo island Korea and which was reported to promote growth and induce systemic resistance in tobacco against leaf soft rot was used (Ham et al., 2009). For experimental use, KUDC1013 was streaked on Tryptic Soy Agar (TSA) plates (Difco Laboratories, USA) and incubated at 30°C for 24 hours. The leaf soft rot pathogen *P. carotovorum* subsp. *carotovorum* was grown in Luria Bertani (LB) agar supplemented with 100 µg/ml ampicillin. For long term storage, bacteria were maintained in 15% (v/v) glycerol at -70°C.

Bacterial cell suspension and heat-treated cells. KUDC1013 was grown 24 hours on TSA plates at 30°C. Cells were scraped off the plate and suspended in a sterile saline solution (0.85% NaCl) adjusted to a final concentration of 10⁸ CFU/ml. KUDC1013 heat-treated cells were obtained by autoclaving and viability of these heat-treated cells was checked by dilution plating of the suspension on TSA. The heat-treated cell suspension was used at the same concentration with living cells. Suspensions were subjected to ISR bioassay as described below.

Extraction and purification of LPS. Lipopolysaccharide containing cell walls were obtained using the method

described by Leeman et al. (1995). Bacterial cells grown on TSA plates were suspended in phosphate buffered saline (10 mM, pH 7.2), centrifuged at 4000 g for 5 min and lyophilized. Lyophilized cells were suspended in 50 mM Tris/HCl, 2 mM EDTA buffer (pH 8.5) and sonicated six times at resonance amplitude for 15 s at 4°C. The sonicated suspension was centrifuged (600 g, 20 min) to remove intact cells and the supernatant was again centrifuged (8,000 g, 60 min) to obtain the crude LPS as a pellet. LPS was purified according to the method of Reznia et al. (2011). LPS was resuspended in PBS and treated with enzymes to eliminate contaminating proteins and nucleic acids. An equal volume of hot (67–75°C) 90% phenol was added with vigorous shaking at 65–70°C for 15 min and the suspension was cooled in ice. The mixture was centrifuged at 8,500 g for 15 min. Sodium acetate at 0.5 M final concentration and 10 volumes of 95% ethanol were added to the supernatant. The samples were stored at -20°C overnight in order to precipitate the LPS and followed by centrifugation at 2,000 g, 4°C for 10 min. The pellets were resuspended in distilled water and an equal amount of chloroform was added followed by shaking in order to remove traces of phenol. The suspension was centrifuged at 2,000 g, 4°C for 10 min and the purified LPS was lyophilized and stored at 4°C.

Flagella extraction. Flagella were prepared as described by Meziane et al. (2005). Bacterial cells grown on TSA were harvested and suspended in 20 mM potassium-phosphate buffer, pH 7.4 (KP buffer) followed by centrifugation at 5,700 g, 5 min. The pellet was resuspended in KP buffer and treated in a homogenizing blender for 60 s to shear off the flagella. Intact cells and cell debris were removed by centrifugation at 23,400 g, 1 h, 4°C and flagella were collected by high speed centrifugation (60,000 g, 4 h, 4°C). The pellet was stored at -20°C.

Isolation of ISR determinant from KUDC1013 cell free supernatant. *O. lupini* KUDC1013 was grown in Tryptic Soy Broth (TSB) on a rotary shaker (180 rpm) at 30°C for 48 h to stationary phase. Cells were eliminated by centrifugation at 8,000 g for 15 min. 300 ml from the obtained supernatant was lyophilized and the resulting powder was suspended in 30 ml sterile distilled water (SDW). Heat-treated supernatant was obtained by autoclaving. The suspensions were subjected to ISR bioassay as described below. The remaining culture filtrate was extracted twice with a double volume of *n*-hexane, ethyl acetate and *n*-butanol (Fig. 1). Extracts were evaporated to dryness in an EYELA model N-1000 rotary evaporator at 50°C. The extracts were dissolved in 10% Dimethylsulfoxide (DMSO, Kanto Chem. Inc., Japan) and were subjected to ISR

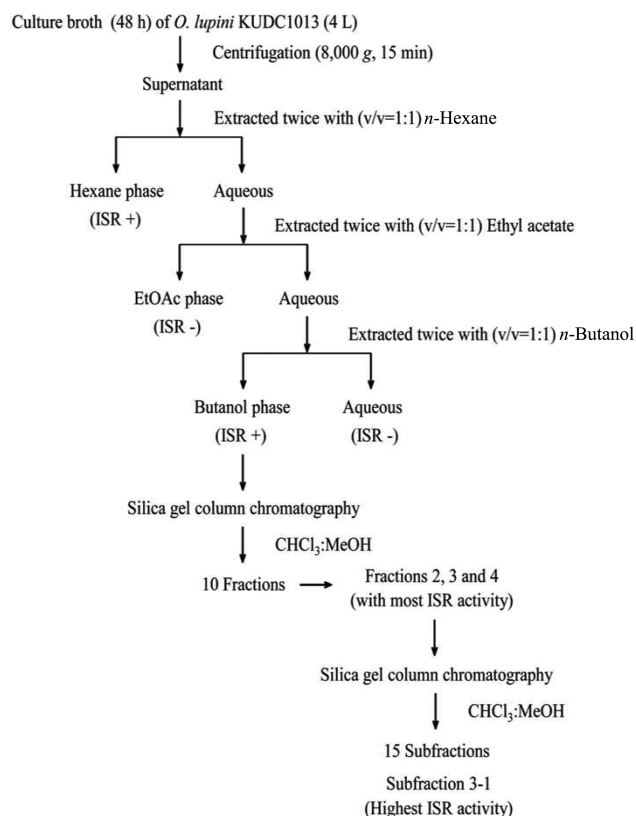


Fig. 1. Extraction procedure and ISR activity of extracts from *O. lupini* KUDC1013. KUDC1013 was grown in Tryptic Soy Broth (4 L) for 48 h and extracted with organic solvents.

bioassay as described below. The extract with highest ISR activity was subjected to silica gel column chromatography. An open glass column (50 mm diameter × 820 mm long) was slurry packed with silica gel in chloroform and washed with two bed volumes of chloroform methanol. The extracts were loaded onto the column and eluted with two bed volumes of a solvent mixture of chloroform and methanol, in which the methanol concentration was increased at each elution step. Each eluate was evaporated and subjected to ISR bioassay. The fraction with the ISR activity was further processed and sub-fractions were tested for their ISR eliciting capacity. The most ISR active sub-fraction was subjected to instrumental analysis. Gas chromatography/mass spectrometry (GC/MS) investigation of the most ISR active sub-fraction was carried out with an Agilent model 7890A-5975C GC/MSD equipped with a capillary column (0.25 ID × 30 m × 0.25 μm film thickness) and electron ionization (EI) as the ion source. The carrier gas is helium and the flow rate is 1.0 ml/min. The column temperature was set at 30°C for 3 min, elevated at 50°C/min to 180°C and by 40°C/min to 200°C. The chemicals present in the sub-fraction were purchased (Sigma Aldrich, Korea) and were tested for ISR elicitation.

Plant material and induced systemic resistance bioassay.

Tobacco (*Nicotiana tabacum*) seeds were surface sterilized by soaking in 1.2% sodium hypochlorite solution for 30 min and washed extensively with SDW. Seeds were allowed to germinate on petri dishes containing half-strength Murashige and Skoog salt (MS) medium containing 1.5% sucrose and 0.8% plant agar, for 2–3 days at 25°C in the absence of light (Murashige and Skoog, 1962). Equally germinated seeds were transferred to 24-well microtitre plate (SPL, Korea), one seed on each well containing 1.5 ml of MS agar media and grown in a growth chamber at 25°C with 14 h/10 h light/dark conditions. One week after transplanting, tobacco plants were inoculated in the root part with 10 μl of different preparations tested for their ISR activity. Control plants were treated with SDW or 10% DMSO as negative controls and 0.5 mM Benzothiadiazole (BTH) as positive control. Seven days after root treatment, four leaves per tobacco seedling were dropped each with 5 μl suspensions of *P. carotovorum* subsp. *carotovorum* (10⁸ CFU/mL). Soft rot symptom was determined by visual examination of leaves 24 h after inoculation. The disease incidence was measured by counting the number of symptomatic leaves per seedling (Ryu et al., 2004). The experiment was repeated three times, three replicates per treatment with 24 plants per replicate.

Statistical analysis. Data were analyzed by analysis of variance (ANOVA) and the means were separated by the least significant difference test using JMP software version 4.04 (SAS Institute, USA). The significance of the effects of the treatments was determined by Duncan's multiple range test ($p = 0.05$).

Results and Discussion

O. lupini KUDC1013 was previously reported to promote the growth of tobacco under laboratory conditions and of pepper under laboratory and field conditions (Ham et al., 2009; Hahm et al., 2012). This rhizobacterium possess plant growth-promoting characteristics including the ability to solubilize phosphate, produce indole-3-acetic acid and siderophore (Ham et al., 2009). Aside from its plant growth-promoting ability, KUDC1013 has been shown to elicit ISR against several pathogens and under greenhouse and field conditions (Ham et al., 2009; Hahm et al., 2012). In this study, factors from KUDC1013 involved in ISR activity were investigated using tobacco as the model plant and *P. carotovorum* subsp. *carotovorum* as the challenging pathogen.

Treatments containing live KUDC1013 cells or heat-treated cells were tested for ISR activity in order to determine if there is any heat stable cell envelop or cell wall related

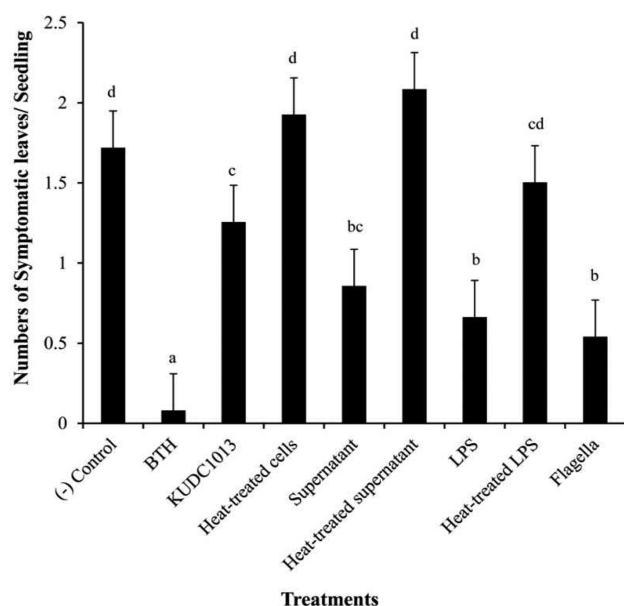


Fig. 2. Resistance induced in tobacco plant by *O. lupini* KUDC1013 and its components against *P. carotovorum* subsp. *carotovorum*. Symptom was expressed as number of symptomatic leaves per seedling. Sterile distilled water (SDW) and 0.5 mM Benzothiadiazole (BTH) were used as negative and positive control, respectively. Vertical bars represent standard errors. Different letters indicate significant differences between treatments according to Duncan's multiple range test at $p = 0.05$. The data are representative of three experiments, three replicates per treatment with 24 plants per replicate.

components that play a major role in ISR elicitation. Results showed that tobacco plants treated with KUDC1013 have lesser symptoms compared with those treated only with sterile distilled water and heat-treated cell preparation did not provide any protection (Fig. 2). Cell wall components such as LPS and flagellin were reported to elicit ISR (Gomez-Gomez and Boller, 2002; Leeman et al., 1995; van Peer and Schippers, 1992). When suspension containing LPS of KUDC1013 was treated in the rhizosphere of tobacco, the disease incidence was lower compared with control (Fig. 2). In certain PGPR strains, bacterial LPS present in the outer membrane of PGPR are the major determinants of ISR. LPS from *Pseudomonas* sp. strain WCS417r induce resistance in radish and carnation to *Fusarium* wilt. The heat-treated cells and purified LPS of WCS417r both triggered ISR in carnation to a level similar to that obtained by treatment with viable bacterial cells (Leeman et al., 1995; van Peer and Schippers, 1992). LPS of *Rhizobium etli* strain G12 induced systemic resistance of potato to infection by cyst nematode *Globodera pallida*. Both living and heat-killed cells of the rhizobacterium elicited protection in potato (Reitz et al., 2000). However, in this study LPS elicited ISR while heat-treated LPS did

not provide any protection (Fig. 2). This suggests that LPS from KUDC1013 could be heat-labile. LPS from KUDC1013 should be further characterized and tested using mutants as it was suggested that different LPS compositions have different resistance responses to plants (Leeman et al., 2005; Whatley et al., 1980).

In addition to LPS, treatment of tobacco roots with flagella preparations of KUDC1013 significantly decreased soft rot symptoms (Fig. 2). Some PGPR mediates ISR through flagella (Gomez-Gomez and Boller, 2002). Bacterial flagellin, the principal structural component of flagella, is recognized in plants by surface receptors containing extracellular leucine-rich repeat (LRR) domain FLS2 (Mendei et al., 2000). Flagellins purified from *Pseudomonas syringae* pv. *glycinea*, an incompatible pathogen for tobacco, induced immune responses in tobacco (Taguchi et al., 2003). Flagella preparations of KUDC1013 elicited ISR against *P. carotovorum* thus; it is interesting to know examine flagella lacking mutant of KUDC1013 for their ability to induce ISR and to determine activation of resistance pathways in tobacco on perception of flagellin from KUDC1013.

A reduction in soft rot disease symptom was observed in plants treated with the crude supernatant obtained after growth of strain KUDC1013 in TSB. Moreover, heat-treated supernatant did not elicit ISR suggesting that the ISR-related metabolite present in the supernatant could be heat labile. To further investigate the metabolites present in KUDC1013 supernatant, it was extracted by a series of organic solvents. ISR bioassay using the organic extracts and remaining aqueous suspension showed that compounds in the n-hexane and n-butanol fractions have ISR eliciting capability with n-butanol having higher protective effect while remaining aqueous suspension and ethyl acetate fractions were impaired of ISR activity (Figs. 1 and 3). The n-butanol extract was further processed through silica gel column chromatography and fractions were subjected to ISR bioassay. The most reduction in soft rot disease incidence was observed in treatment with subfraction 3-1 (Fig. 1). Gas chromatography/Mass spectrometry (GC/MS) analysis revealed that compounds were present in subfraction 3-1 namely; N-methyl-2-pyrrolidone, phenylacetic acid, 2,4-d-t-butylphenol, linoleic acid, oleoyl chloride and 1-hexadecene (Fig. 4). ISR bioassay using these compounds showed that among them, linoleic acid, 1-hexadecene and phenylacetic acid induced systemic resistance against leaf soft rot pathogen (Fig. 5).

Phenyl acetic acid (PAA) at 1 mM and 0.1 mM induced systemic resistance of tobacco against *P. carotovorum* (Fig. 5). PAA is known as an auxinomimetic compound with the ability to stimulate cell enlargement similar to a plant growth promoting hormone IAA (Fries, 1977; Milborrow et al., 1975). PAA is produced by both plants (Isogai et al.,

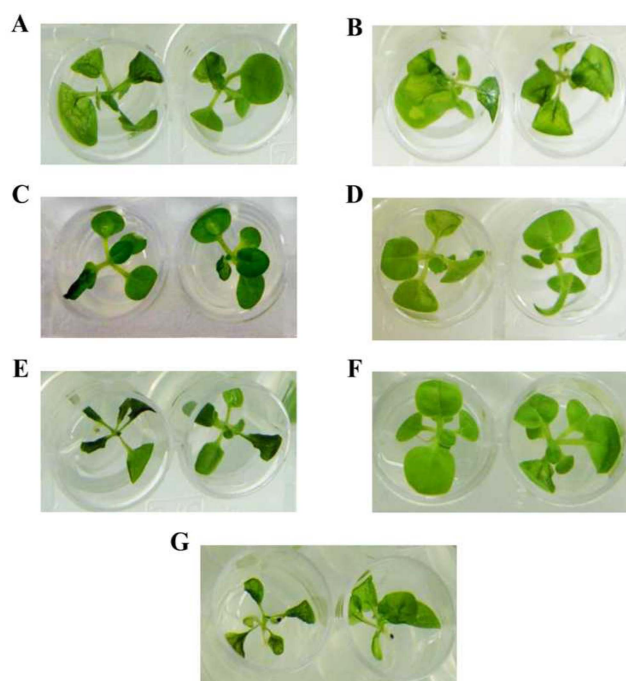


Fig. 3. Effect of extracts of KUDC1013 on soft rot symptoms on tobacco leaves. Roots of seedlings were inoculated with Sterile distilled water (SDW) (A), 10% Dimethylsulfoxide (DMSO) (B), 0.5 mM Benzothiadiazole (BTH) (C), *n*-Hexane (D), Ethyl acetate (E), *n*-Butanol (F), and Aqueous (G) extracts. SDW and 10% DMSO were used as negative controls and BTH as positive control. One week after treatment, plant leaves were challenged with soft rot pathogen, as described in Materials and Methods. Photographs were taken 24 h after inoculation with *P. carotovorum*. The photographs are representative of three independent experiments with at least 72 seedlings per treatment.

1967) and by bacteria (Hwang et al., 2001; Sarwar et al., 1995; Somers et al., 2005). PAA showed inhibitory activity against zoospore germination and hyphal growth of *Phytophthora capsici* (Lee et al., 2004) and antifungal activity against the dry rot causative pathogen *Gibberella pulicaris*, and was shown to suppress dry rot infection of wounded potatoes (Slininger et al., 2004).

Induction of systemic resistance was also observed in tobacco plants treated with 0.01 mM 1-hexadecene. Hexadecene is a hydrocarbon commonly reported in seaweeds (Zakaria et al., 2011). Hexadecene observed in the crude extract of the microalgae *Spirulina platensis* showed antimicrobial activity against pathogenic bacteria (Kumar et al., 2011). Also, hexadecene was isolated from the liquid culture of the biocontrol fungus *Trichoderma harzianum* strain FA1132 (Siddiquee et al., 2012).

Linoleic acid (LA) is a fatty acid found in many natural sources including vegetable oils from safflower, sunflower and corn oils and is produced by some bacteria (Alonso et al., 2003; Chin et al., 1992; Macouzet et al., 2009). It is

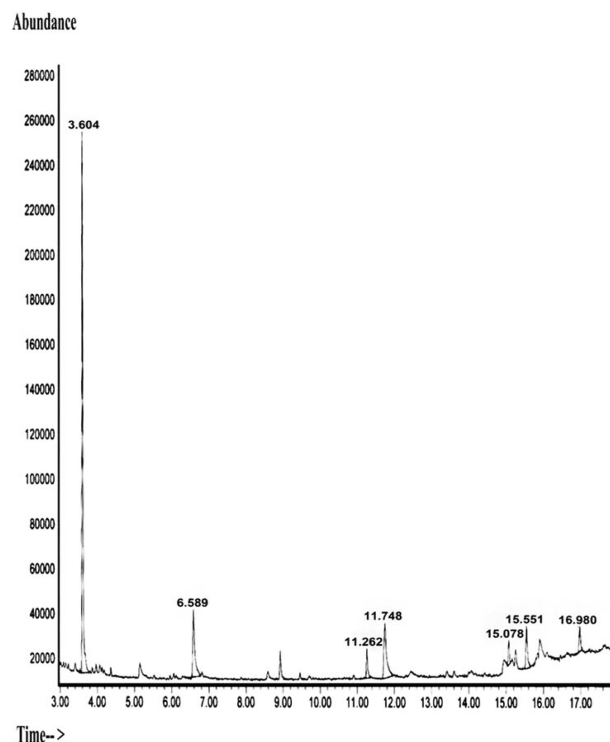


Fig. 4. Chromatogram obtained in Gas Chromatography and Mass Spectrometry analysis for identification of the ISR determinant(s) of *O. lupini* KUDC1013 present in the ISR-active butanol subfraction 3-1.

considered as a biologically beneficial functional lipid because of its many nutraceutical purposes (Ogawa et al., 2005). Kabara et al. (1972) demonstrated that linoleic acid possessed biocidal effects against gram positive bacteria. In this study, treatment of tobacco roots with 1 mM LA caused death to plants while it significantly decreased the soft rot symptoms at 0.1 mM and 0.01 mM (Fig. 5). This suggests that LA, at higher concentration is toxic to tobacco and is possibly more effective in eliciting ISR at lower concentrations. Linoleic acid inhibited the mycelial growth of *Alternaria solani*, *Fusarium oxysporum* f. sp. *lycopersici*, and *F. oxysporum* f. sp. *cucumerinum* at 2 mM and exhibited an antifungal activity against *Crinipellis perniciosa* at the lower level of 0.1 mM (Liu et al., 2008; Walters et al., 2004).

Some biologically active metabolites play a role as elicitors of ISR at low concentration but showed antimicrobial activity at higher concentration (Rohilla et al. 2002; Tosi and Zizzerini, 2000). PAA, 1-hexadecene and LA, at concentration tested for direct antibiosis (0.01 mM–2.0 mM), did not exhibit any direct antimicrobial activity against the pathogen *in vitro* (data not shown). Different ISR-active compounds elicit ISR at different concentrations. The ISR-active metabolite 4-aminocarbonyl phenylacetate from

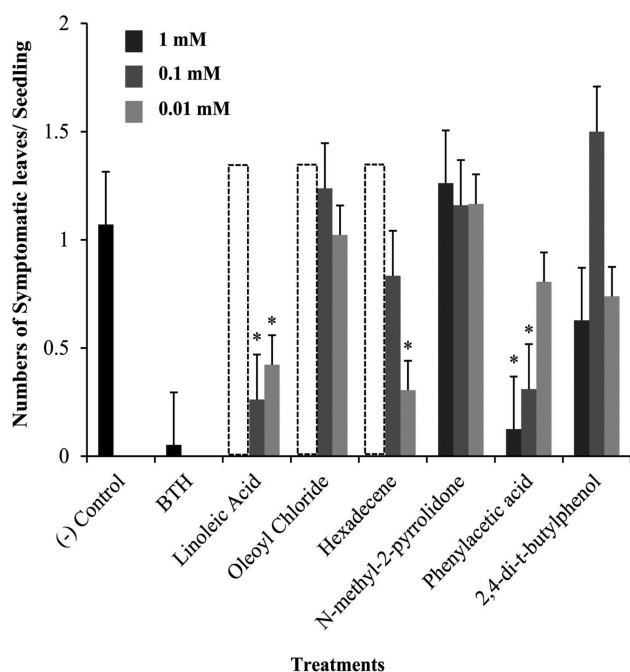


Fig. 5. Influence of root treatment with chemicals present in n-butanol subfraction 3-1 on the number of symptomatic leaves on tobacco after inoculation with soft rot pathogen *P. carotovorum*. Sterile distilled water (SDW) and 0.5 mM Benzothiadiazole (BTH) were used as negative and positive control, respectively. The highest ISR eliciting subfraction 3-1 was subjected to GC/MS analysis and chemicals present were purchased and subjected to ISR bioassay. The data are representative of three experiments, three replicates per treatment with 24 plants per replicate. Blank bars mean plants died after treatment. Vertical bar represents standard errors. *Indicates significantly different from negative control at LSD ($p = 0.05$).

Pseudomonas chlororaphis O6 applied at 68.0 mM elicited ISR activity against wildfire pathogen at a level similar to 1.0 mM salicylic acid (Park et al., 2008). The ISR effect of butyl-2-pyrrolidone-5-carboxylate (BPC) from *Klebsiella oxytoca* C1036 was observed at 12 mM (Park et al., 2009). In this study PAA elicited ISR at 1.0 mM and 0.1 mM, 1-hexadecene at 0.01 mM while LA elicited ISR at concentration of 0.1 and 0.01 mM (Fig. 5).

These results demonstrated the various determinants of KUDC1013 involved in ISR activity. The recognition of KUDC1013 cell wall components, LPS and flagella led to the induction of systemic resistance of tobacco against soft rot disease. Metabolites present in the culture supernatant of KUDC1013 that elicit ISR against soft rot disease were identified as PAA, 1-hexadecene and LA. Application of these compounds activated the disease resistance of tobacco against soft rot. To the author's knowledge, this is the first report of PAA, 1-hexadecene and LA as ISR-related metabolites from KUDC1013.

The present work shows that *O. lupini* KUDC1013 has

multiple factors involved in the induction of systemic resistance. Further studies are therefore necessary to elucidate the mode of actions and the physiological functions of KUDC1013 determinants in order to maximize their biological control potential.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2011-0011565).

References

- Alonso, L., Cuesta, E. P. and Gilliland, S. E. 2003. Production of free conjugated linoleic acid by *Lactobacillus acidophilus* and *Lactobacillus casei* of human intestinal origin. *J. Dairy Sci.* 86:1941–1946.
- Babalola, O. O. 2010. Beneficial bacteria of agricultural importance. *Biotechnol. Lett.* 32:1559–1570.
- Cameron, R.K., Dixon, R. and Lamb, C. 1994. Biologically induced systemic acquired resistance in *Arabidopsis thaliana*. *Plant J.* 5:715–725.
- Chin, S. F., Liu, W., Storkson, J. M., Ha, Y. L. and Pariza, M. W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Comp. Anal.* 5:185–197.
- Chin-A-Woeng, T. F. C., Bloemberg, G. V., van der Bij, A. J., van der Drift, K. M. G. M., Schripsema, J., Kroon, B., Scheffer R. J., Keel, C., Bakker, P. A. H. M., Tichy, H. V., de Bruijn, F. J. Thomas-Oaters, J. E. and Lugtenberg, B. J. J. 1998. Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Mol. Plant-Microbe Interact.* 11:1069–77.
- Dunne, C., Möenne-Loccoz, Y., McCarthy, J., Higgins, P., Powell, J., Dowling, D. N. and O'Gara, F. 1998. Combining proteolytic and phloroglucinol-producing bacteria for improved control of *Pythium*-mediated damping-off of sugar beet. *Plant Pathol.* 47:299–307.
- Fries, L. 1977. Growth regulating effects of phenylacetic acid and phydroxy-phenylacetic acid on *Fucus spiralis* L. (Phaeophyceae, Fucales) in axenic culture. *Phycology* 16:451–455.
- Gomez-Gomez, L. and Boller, T. 2002. Flagellin perception: a paradigm for innate immunity. *Trends Plant Sci.* 7:251–256.
- Haas, D. and Keel, C. 2003. Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu. Rev. Phytopathol.* 41:117–53.
- Hahm, M. S., Sumayo, M., Hwang, Y. J., Jeon, S. A., Park, S. J., Lee, J. Y., Ahn, J. H., Kim, B. S. and Ghim, S.-Y. 2012. Biological control and plant growth promoting capacity of rhizobacteria on pepper under greenhouse and field conditions. *J. Microbiol.* 50:380–385.

- Ham, M. S., Park, Y. M., Sung, H. R., Sumayo, M., Ryu, C. M., Park, S. H. and Ghim, S.-Y. 2009. Characterization of rhizobacteria isolated from family Solanaceae plants in Dokdo island. *Kor. J. Microbiol. Biotechnol.* 37:110–117.
- Han, S. H., Anderson, J. A., Yang, K. W., Cho, B. H., Kim, K. Y., Lee, M. C., Kim, Y. H. and Kim, Y. C. 2006. Multiple determinants influence root colonization and induction of induced systemic resistance by *Pseudomonas chlororaphis* O6. *Mol. Plant Pathol.* 7:463–472.
- Hwang, B. K., Lim, S. W., Kim, B. S., Lee, J. Y. and Moon, S. S. 2001. Isolation and *in vivo* and *in vitro* antifungal activity of phenylacetic acid and sodium phenylacetate from *Streptomyces humidus*. *Appl. Environ. Microbiol.* 67:3739–3745.
- Isogai, Y., Okamoto, T. and Koizumi, T. 1967. Isolation of indole-3-acetamide, 2-phenylacetamide and indole-3-carboxaldehyde from etiolated seedlings of *Phaseolus*. *Chem. Pharm. Bull. Tokyo.* 15:151–158.
- Jetiyanan, K. and Kloepper, J. W. 2002. Mixtures of plant-growth promoting rhizobacteria for induction of systemic resistance against multiple diseases. *Biol. Control* 24:285–291.
- Kamilova, F., Validov, S., Azarova, T., Mulders, I. and Lugtenberg, B. 2005. Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environ. Microbiol.* 7:1809–17.
- Kloepper, J. W., Tuzun, S. and Kuc, J. A. 1992. Proposed definitions related to induced disease resistance. *Biocontrol Sci. Tech.* 2:349–351.
- Kumar, V., Bhatnagar, A. K. and Srivastava, J. N. 2011. Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compound. *J. Med. Plants. Res.* 5:7043–7048.
- Lee, J. Y., Kim, H. S., Kim, K. D. and Hwang, B. K. 2004. *In vitro* anti-oomycete and *in vivo* control efficacy of phenylacetic acid against *Phytophthora capsici*. *Plant Pathol. J.* 20:177–183.
- Leeman, M., van Pelt, J. A., Den Ouden, F. M., Heinsbroek, M., Bakker, P. A. H. M. and Schippers, B. 1995. Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. *Phytopathology* 85:1021–1027.
- Liu, S., Weibin, R., Jing, L., Hua, X., Jingan, W., Yubao, G. and Jingguo, W. 2008. Biological control of phytopathogenic fungi by fatty acids. *Mycopathologia* 166:93–102.
- Lugtenberg, B. and Kamilalova, F. 2009. Plant growth promoting rhizobacteria. *Annu. Rev. Microbiol.* 63:541–556.
- Macouzet, M., Lee, B. H. and Robert, N. 2009. Production of conjugated linoleic acid by probiotic *Lactobacillus acidophilus* La-5. *J. Appl. Microbiol.* 106:1886–1891.
- Meindl, T., Boller, T. and Felix, G. 2000. The bacterial elicitor flagellin activates its receptor in tomato cells according to the address-message concept. *Plant Cell* 12:1783–1794.
- Meziane, H., van der Sluis, I., van Loon, L. C., Höfte, M. and Bakker, P. A. H. M. 2005. Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. *Mol. Plant Pathol.* 6:177–185.
- Milborrow, B. V., Purse, J. G. and Wightman, F. 1975. On the auxin activity of phenylacetic acid. *Ann. Bot.* 39:1143–1146.
- Ogawa, J., Kishino, S., Ando, A., Sugimoto, S., Mihara, K. and Shimizu, S. 2005. Production of conjugated fatty acids by lactic acid bacteria. *J. Biosci. Bioeng.* 100:355–364.
- Ongena, M., Jourdan, E., Schäfer, M., Kech, C., Budzikiewicz, H., Luxen, A. and Thonart, P. 2005. Isolation of an N-alkylated benzylamine derivative from *Pseudomonas putida* BTP1 as elicitor of induced systemic resistance in bean. *Mol. Plant-Microbe Interact.* 18:562–569.
- Park, M. R., Kim, Y. C., Park, J. Y., Han, S. H., Kim, K. Y., Lee, S. W. and Kim, I. S. 2008. Identification of an ISR-related metabolite produced by *Pseudomonas chlororaphis* O6 against wild-fire pathogen *Pseudomonas syringae* pv. *tabaci* in tobacco. *J. Microbiol. Biotechnol.* 18:1659–1662.
- Park, M. R., Kim, Y. C., Lee, S. and Kim, I. S. 2009. Identification of an ISR-related metabolite produced by rhizobacterium *Klebsiella oxytoca* C1036 active against soft-rot disease pathogen in tobacco. *Pest Manag. Sci.* 65:1114–1117.
- Reitz, M., Rudolph, K., Schröder, I., Hoffmann-Hergarten, S., Hallmann, J. and Sikora R. A. 2000. Lipopolysaccharides of *Rhizobium etli* strain G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode *Globodera pallida*. *Appl. Environ. Microbiol.* 66:3515–3518.
- Rezania, S., Amirmozaffari, N., Bahman Tabarraei, B., Jedditehrani, M., Zarei, O., Alizadeh, R., Masjedian, F. and Amir Hassan Zarnani, A. H. 2011. Extraction, purification and characterization of lipopolysaccharide from *Escherichia coli* and *Salmonella typhi*. *Avicenna J. Med. Biotech.* 3:3–9.
- Rohilla, R., Singh, U.S. and Singh, R. L. 2002. Mode of action of acibenzolar-S-methyl against sheath blight of rice, caused by *Rhizoctonia solani* Kühn. *Pest. Manag. Sci.* 58:63–69.
- Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Kloepper, J. W. and Paré, P. W. 2004. Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol.* 134:1017–1026.
- Ryu, C. M., Kim, J., Choi, O., Park, S. Y., Park, S. H. and Park, C. S. 2005. Nature of a root-associated *Paenibacillus polymyxa* from field-grown winter barley in Korea. *J. Microbiol. Biotechnol.* 15:984–991.
- Sarwar, M. and Franckenberger, W. T. Jr. 1995. Fate of lphenylalanine in soil and its effect on plant growth. *Soil Sci. Soc. Amer. J.* 59:1625–1630.
- Siddiquee, S. Cheond, B. E., Taslima, K. Kausar, H. and Hasan M. M. 2012. Separation and identification of volatile compounds from liquid cultures of *Trichoderma harzianum* by GC-MS using three different capillary columns. *J. Chromatogr. Sci.* 50:358–367.
- Slininger, P. J., Burkhead, K. D. and Schisler, D. A. 2004. Antifungal and sprout regulatory bioactivities of phenylacetic acid, indole-3-acetic acid, and tyrosol isolated from the potato dry rot suppressive bacterium *Enterobacter cloacae* S11:T:07. *J. Ind. Microbiol. Biotechnol.* 31:517–524.
- Somers, E., Ptacek, D. Gysegom, P., Srinivasan, M. and Vanderleyden, J. 2005. *Azospirillum brasilense* produces the auxin-like phenylacetic acid by using the key enzyme for indole-3-acetic acid biosynthesis. *Appl. Environ. Microbiol.* 71:1803–1810.

- Taguchi, F., Shimizu, R., Nakajima, R., Toyoda, K., Shiraishi, T. and Ichinose, Y. 2003. Differential effects of flagellins from *Pseudomonas syringae* pv. *tabaci*, tomato and *glycinea* on plant defense response. *Plant Physiol. Biochem.* 41:165–174.
- Tosi, L. and Zizzerini, A. 2000. Interactions between *Plasmopara helianthi*, *Glomus mosseae* and two plant activators in sunower plants. *Eur. J. Plant Pathol.* 106:735–744.
- van Loon, L. C., Bakker, P. A. H. M. and Pieterse, C. M. J. 1998. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol.* 36:453–483.
- van Peer, R. and Schippers, B. 1992. Lipopolysaccharides of plant growth promoting *Pseudomonas* spp. strain WCS417r induce resistance in carnation to *Fusarium* wilt. *Neth. J. Pl. Path.* 98: 129–139.
- van Peer, R., Niemann, G. J. and Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS417r. *Phytopathology* 81:728–34.
- Walters, D., Raynor, L., Mitchell, A., Walker, R. and Walker, K. 2004. Antifungal activities of four fatty acids against plant pathogenic fungi. *Mycopathologia* 157:87–90.
- Wei, G., Kloepper, J. W. and Tuzun, S. 1991. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth promoting rhizobacteria. *Phytopathology* 81:1508–1512.
- Whatley, M. H., Hunter, N., Cantrell, M. A., Hendrick, C. A., Keegstra, K. and Sequeira, L. 1980. Lipopolysaccharide composition of the wilt pathogen, *Pseudomonas solanacearum*: correlation with hypersensitive response in tobacco. *Plant Physiol.* 65:557–559.
- Zakaria, N. A., Ibrahim, D., Fariza Shaida, S. F. and Afifah Supardy, N. A. 2011. Phytochemical composition and antibacterial potential of hexane extract from Malaysian red algae, *Acanthophora spicifera* (Vahl) Borgesen. *World Appl. Sci. J.* 15: 496–501.
- Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E. J., Jones, J. D. G., Felix, G. and Boller, T. 2004. Bacterial disease resistance in *Arabidopsis* through flagellin perception. *Nature* 428:764–767.