

## 세균 병학

**B-01 Induction of Systemic Resistance in Watermelon against Gummy stem rot by Plant Growth Promoting Rhizobacteria.** Yong Hoon Lee<sup>1</sup>, Wang Hyu Lee<sup>2</sup>, Hyeong Kwon Shim<sup>1</sup>, Du Ku Lee<sup>1</sup>. <sup>1</sup>Division of Plant Environment, National Honam Agricultural Experiment Station, RDA, Iksan, Chonbuk, Korea 570-080. <sup>2</sup>Faculty of Biological Resources Science, Chonbuk National University, Chonju, Korea 560-756.

The selected PGPR strains, WR8-3 (*P. fluorescens*), WR8-6 (*P. putida*), WR9-9 (*P. fluorescens*), WR9-11 (*Pseudomonas* sp.), and WR9-16 (*P. putida*) isolated from the rhizosphere of watermelon were tested on watermelon growth promotion and resistance induction to *D. bryoniae*. The strain WR8-3 and WR9-16 significantly increased stem length of watermelon. And there was a little increase in leaf area, fresh weight and root length, when strain WR8-3, WR9-9 and WR9-16 were treated. Generally, the seed treatment showed a little increase than the soil drench, but there was no significant difference. WR9-9, WR9-11, and WR9-16 significantly reduced lesion diameter. In general, seed treatment with the PGPR strains reduced the MLA than soil drench, but, there was no significant difference. Increasing the rhizosphere population density up to approximately  $10^6$  cfu/ml resulted in a decrease of MLA. Resistance could be induced by treating the strains, WR9-9, WR9-11 and WR9-16 in all of four watermelon cultivars tested. The strains introduced on seed followed a similar pattern with a rapid decrease from planting day to 1 week after planting, but the population densities maintained between log 5 and 8 cfu/g of root to 4 weeks after planting. Generally, no or very weak *in vitro* antagonism was observed excepting WR9-11. No rifampicin resistant bacteria were detected in the stems or leaves, which indicated that the bacterium and the fungus remained spatially separated during the experiment.

**B-02 Factors Relating to ISR in Watermelon by Plant Growth Promoting Rhizobacteria.** Yong Hoon Lee<sup>1</sup>, Wang Hyu Lee<sup>2</sup>, Du Ku Lee<sup>1</sup>, Hyeong Kwon Shim<sup>1</sup>. <sup>1</sup>Division of Plant Environment, National Honam Agricultural Experiment Station, RDA, Iksan, Chonbuk, Korea 570-080. <sup>2</sup>Faculty of Biological Resources Science, Chonbuk National University, Chonju, Korea 560-756.

The PGPR strains which showed ISR to gummy stem rot in watermelon were investigated on their ISR related characteristics. All of the strains produced fluorescent pigments on KB, and the level of pyoverdine production was greatest in the strain of WR8-6. But the iron-binding ability on CAS was observed only in the WR8-3 and WM9-16 strain. The percentage of pyoverdine was reversely related to the concentration of  $FeCl_3$ . The siderophore-negative mutant WR8-3m and WR9-16m were repressed pyoverdine production, and couldn't inhibit mycelial growth on KB, and promote the watermelon growth and induce resistance. WM8-3 and WM9-16 inhibited more greatly the growth of *D. bryoniae* at low concentration of  $FeCl_3$  on KB. But, no antagonism against the pathogen was observed according to the increase of iron concentration. The strain WR9-11 showed antagonism from 0 to  $1,000 \mu M$  of  $FeCl_3$ . The crude LPS of each strain couldn't reduce MLA, which means that the crude LPS of the strains was not the major determinants. The induced resistance by the strain WR9-11, which produced HCN and pyochelin, and showed strong antifungal activity, was estimated as the production of HCN. SA production did not identified in all of the strains. The ISR mechanism by the strain WR8-06 and WM9-09 did not identified, so the other mechanism which did not experimented here might be involved.

**B-03 Comparison of *In Vitro* and *In Vivo* Tests for Selection of Antagonistic Bacteria to Suppress Phytophthora Blight of Pepper.** Ki Deok Kim, Sung Hwan Chang, and Byung Kook Hwang. Department of Agricultural Biology, Korea University, Seoul 136-701, Korea.

*In vitro* tests associated with pepper seeds for screening bacteria antagonistic to *Phytophthora capsici* were developed and reported previously. Therefore, in this study, we attempted to compare the effectiveness of antagonistic bacteria selected from *in vitro* tests with *in vivo* tests using pepper plants. Germinated pepper seeds (cv. Nokwang) were sown in a potting mixture incorporated with antagonistic bacterial cells (1ml of cells of O.D. 0.5 at 640 nm per gram of the mixture). Three-week-old plants were then transplanted into steam-sterilized soils. The plants were hole-inoculated with zoospores of *P. capsici* (25 zoospores per gram of soil) 2 weeks after transplanting. Disease severity with a 0 (no visual symptom) and 5 (plant dead) scale was evaluated everyday for 16 to 20 days after inoculation. AUDPC for disease severity and final disease incidence were also determined. Among 64 bacterial strains tested, GC-B19, GK-B15, GK-B25, MW-B02, OA-B26, OA-B36, OA-B37, OA-B65, and RC-B39 were determined to be effective bacterial strains for suppression of Phytophthora blight of peppers by *P. capsici*. The strains GC-B19, GK-B15, GK-B25, OA-B26, OA-B36, OA-B37, and RC-B39 also proved to be effective strains for inhibition of seed rot by *P. capsici* in *in vitro* tests. Since *in vivo* tests to select antagonistic bacteria require much time and efforts, pre-selection of effective bacteria through *in vitro* tests with pepper seeds could reduce time and efforts.

**B-04 Effects of charcoal and charcoal wood extracts on the population change of plant growth promoting bacteria, and the hot pepper(*Capsicum annum* L.) plant growth, and root and fruit development.** Youn Su Lee<sup>1</sup> and Sung Jae Lee<sup>2</sup>. <sup>1</sup>Div. of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, Korea 200-092. <sup>2</sup>Forest Research Institute, Kangwon Province, Chuncheon, Korea 218-5

A charcoal is composed of 80-90% of carbon, and the rest of it composed mainly of oxygen, hydrogen, and inorganic substances. The major characteristic of a charcoal is its large interior surface area of 200-300m<sup>2</sup>, which makes it highly porous and absorptive. A charcoal can be useful not only for soil amendment materials but also for habitats of various beneficial microorganism due to its porous and absorptive characteristics. It is generally well known that use of charcoal reduces soil-born plant pathogen populations and increases plant growth. A charcoal also neutralize the acidic soils by mediating the soil pH. In recent studies, charcoal wood extracts have been also known to promote plant growth or reduces plant pathogens on the soil. In the study, we evaluated the effects of charcoal and/or charcoal wood extracts in combination with or without plant growth promoting bacteria on hot pepper plant growth, fruit and root developments. Effects of charcoal and charcoal wood extracts on the bacterial populations were also observed. Here, we are reporting the effects of charcoal and charcoal wood extracts on the population change of plant growth promoting bacteria, and the hot pepper(*Capsicum annum* L.) plant growth, and root and fruit development.

**B-05 Selection of plant growth promoting *Pseudomonas* species for several vegetable crops.** Youn Su Lee. Div. of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-092, Korea

Among *Pseudomonas* isolates from various sources, a *Pseudomonas* species was selected and tested for its plant growth promoting effect on several vegetable crops. As a result, the isolate was found to be effective for growth promotion in vegetable crops including *Lactuca sativa* L. *Daucus carota* var. *sativa* DC. *Allium cepa* L. *Allium fistulosum* L. *Brassica campestris*, *Brassica oleracea* var. *capitata* L. and *Raphanus sativus* L. Even though this result definitely requires further tests, both in the field and *in vitro*, the result indicated high possibility of commercialization of the isolate.

**B-06 *Xanthomonas oryzae* pv. *oryzae* genome: Construction of BAC library and Physical mapping.** Hee-Wan Kang<sup>1</sup>, Dong-Suk Park<sup>1</sup>, In-Cheol Park<sup>1</sup>, Yong-Hwan Kim<sup>2</sup>, Young-Jin Park<sup>1</sup>, Sung-Ho Choi<sup>3</sup>, Byoung-Moo Lee<sup>1</sup>, Seung-Joo Go<sup>1</sup>, and Moo-Young Eun<sup>2</sup> <sup>1</sup>Division of Molecular Genetics, National Institute of Agri. Science & Technology, Suwon 441-707, Korea <sup>2</sup>Division of Cytogenetics, National Institute of Agri. Science & Technology, Suwon 441-707, Korea <sup>3</sup>Division of Plant pathology, National Institute of Agri. Science & Technology, Suwon 441-707, Korea

We initiated *Xanthomonas oryzae* pv. *oryzae* genome project which is targeted for complete genome sequencing. Bacterial artificial chromosome (BAC) library was constructed from the genomic DNA (Korean isolate: Xoo85) partially restricted with *Hind* III using PIndigoBac536 and pBACwch vectors. BAC library contains 3,000 clones with an average insert size of 120 kb providing about 60 genome equivalents based on an estimated genome size of 7 Mbp. BAC contig assembly was constructed with restriction enzyme digest fingerprinting using *Hind* III and *Msp* I. BAC colonies was arrayed on membrane filters with high-density (1,500 BAC colonies per a filter) using Biomtek 2000. Known gene probes in *Xanthomonas oryzae* pv. *oryzae* genome were marked on them by hybridizing to BAC colony filter arrays. Based on the BAC fingerprinting and BAC positions of DNA markers, physical map for the *Xanthomonas oryzae* pv. *oryzae* genome will be discussed.

**B-07 Comparative Analyses of Pathogenicity-related Genes in Korean Strains and Japanese Strains of *Pseudomonas syringae* pv. *actinidiae* Causing Bacterial Canker of Kiwifruit.** Jae Sung Jung<sup>1</sup>, Hyo Shim Han<sup>1</sup>, and Young Jin Koh<sup>2</sup>. <sup>1</sup>Department of Biology, <sup>2</sup>Department of Applied Biology, Sunchon National University, Sunchon, Chonnam 540-742, Korea

To investigate phylogenetic relationships between Korean and Japanese strains, several phytotoxin genes involved in the pathogenicity of *Pseudomonas syringae* pv. *actinidiae* were analyzed. Those of thirty-one Korean strains collected from the major kiwifruit cultivation areas in Korea were compared with those of ten Japanese strains including strain KW11, the type strain of this pathovar. *P. syringae* pv. *actinidiae*, responsible for canker or leaf spot on actinidia plants, is known as one of the phaseolotoxin producers and possesses phaseolotoxin-resistant ornithine carbamoyltransferase (ROCT) which confers resistance to the toxin. Even though the genes for cyclic lipodepsinonapeptides were not detected in any strain of *P. syringae* pv. *actinidiae* collected from both countries, phaseolotoxin and ROCT genes were detected in all Japanese strains including the type-strain KW11 but not in Korean strains. Instead of phaseolotoxin gene cluster, however, coronatine biosynthetic gene cluster was detected in all Korean strains with different geographic origins. The results suggested that Korean strains and Japanese strains of *P. syringae* pv. *actinidiae* might have different phylogenetic origins.

**B-08 Bacterial Soft Rot of Chinese Mustard(*Brassica juncea* L.) by *Erwinia carotovora* subsp. *carotovora* and It's Biological Control.** Ki Suk Doh, Ji Tae Kim, Sung Won Choi, Kee Hyun Choi, and Jae Ho Lee. Green Biotech Ltd., 687-2, Sangisug-ri, Kyoha-myun, Paju-city, Kyungki-do, 413-830 Korea.

Occurrence of soft rots was observed on Chinese mustard that was massively grown in Paju-city, Kyungki-do, Korea. The symptoms was appeared as water-soaked lesions on the near site wounded by harvest. The casual organism isolated from the lesions was identified as *Erwinia carotovora* subsp. *carotovora* based on the physiological and chemical characteristics, and on the results of the Biolog Program (Biolog Inc., U.S.A.). Through field test, *Bacillus polymyxa* AC-1 and *Bacillus subtilis* GB-0365 was selected as biological control agents against soft rots. The control value of *Bacillus polymyxa* AC-1 (New-Hinnara, Green Biotech) and *Bacillus subtilis* GB-0365 (Green-all G, Green Biotech) were 62.2%, 33.3% respectively.

**B-09 A two-component sensor kinase (GacS) is involved in production of quorum sensing factors, secondary metabolites, and competition in the root-colonizing isolate *Pseudomonas chlororaphis* O6.** Beom-Ryong Kang, and Young-Cheol Kim. Applied Plant Science Division and Institute of Biotechnology, College of Agriculture, Chonnam National University, Kwangju 500-757, Korea

*P. chlororaphis* O6 is a biological control bacterium that inhibits several fungal pathogens and produces phenazines, hydrogen cyanide (HCN), and protease. The overproduction of phenazines by this bacterium decreased seed germination and growth of wheat. To study the role of phenazines in biocontrol mechanisms, several phenazine mutants, including negative mutants (L21, N7) and an overexpressed mutant (org), were isolated by Tn5-lux transposon mutagenesis. The *gacS* gene, encoding a sensor kinase, from wild type complemented the L21 mutant although the Tn5 insertion in this mutant was in another *ppx*-like gene, encoding exopolyphosphatase. Complementation with *gacS* restored wild-type properties: production of phenazines, HCN, and homoserine lactone (HSL). To investigate the role of *gacS* in production of secondary metabolites and HSL, we isolated *gacS* mutants by marker exchange mutagenesis. The O6 *gacS* mutants did not produce phenazine, protease, and HSL. The *gacS* mutants also decreased in production of catalase and decreased colonization ability in presence of other microbes. Using *gacS* mutant and confocal laser microscopic technique, we directly demonstrated production of the phenazine in root surface. Our findings confirm that production of several secondary metabolites and HSL formation is regulated by a *gacS/gacA* two-component regulatory system.

**B-10 Control of phytoplasma infecting chrysanthemum (*Dendranthema grandiflorum*).** Bong Nam Chung\*, Gug Seoun Choi, Yong Mun Choi and Doo Won Lee. Horticultural Environment div. National Horticultural Research Institute, RDA, Suwon, 440-310, Korea

Aster Yellows phytoplasma infecting chrysanthemums showed typical phytoplasma infection symptoms of stunting, rosette, excessive branching, and death of plants. Spraying infected chrysanthemums with oxytetracycline in distilled water at a concentration of 0.1g, 0.2g, 0.3g, 0.4g, 0.8g and 1.2g per liter at three days intervals for one month provided temporal recovery from infection. Chrysanthemums sprayed with oxytetracycline concentration of above 0.4g per liter showed distinguished results compared with the not treated ones. But chrysanthemums treated with oxytetracycline concentration of above 0.8g per liter showed damage from the chemical. But, One month after last treatment, chrysanthemums sprayed with oxytetracycline concentration above 0.4g per liter showed lengthening of internode and expanding of their leaves. Examination of ultrathin sections of oxytetracycline treated leaf midribs with electron microscope did not reveal any phytoplasma bodies. But, about two months after last treatment, newly sprouted leaves showed phytoplasma infection symptom of leaf stunting and shortening of internode. We concluded that the recovery from infection was caused by incomplete elimination of phytoplasma bodies and the complete elimination of phytoplasma from chrysanthemum infected with phytoplasma by oxytetracycline spray was very difficult.

**B-11 Efficient Inoculation Methods for Bacterial Soft Rot of Chinese cabbage.**  
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Bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* is one of the most important diseases on Chinese cabbage, *Brassica campestris* subsp. *napus* var. *peckinensis*. It brings huge economic loss of Chinese cabbage production every year in Korea. Induction of soft rot disease on Chinese cabbage by artificial inoculation is very important for many areas: in evaluation of disease resistance of breeding lines, in evaluation of chemical or biological pesticides, in study of host-pathogen interaction. We developed very efficient inoculation methods inducing soft rot disease uniformly and consistently on Chinese cabbage. Spraying bacterial suspension of *Erwinia carotovora* subsp. *carotovora* (Ecc) mixed with 0.2 % Silwet L-77 (OSI Specialties, West Virginia, USA) generated soft rot on Chinese cabbage very well even without maintain the inoculated plants in a moisture chamber. The disease did not developed by spraying of bacterial suspension without Silwet L-77. Mineral oil also helps induction of the disease. Injection of Ecc suspension mixed with mineral oil into midrib of Chinese cabbage generated soft rot symptoms much more consistently than injection of just bacterial suspension. The disease was developed every plants by pouring of the mixture on Chinese cabbage whereas none of the plants showed symptoms of soft rot by pouring of just bacterial suspension. The methods developed in this study will be useful tools in many areas working with bacterial soft rot of Chinese cabbage, and usage of Silwet L-77 or mineral oil may applicable to other soft rot diseases.

**B-12 Occurrence Ecology on Witches'-broom Disease of *Oenanthe javanica* DC. and Diagnosis of Fluorescence Microscopy Using Image Processing.**

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This study was conducted to examine and compare the occurrence aspects of the water dropwort (*Oenanthe javanica* DC.) witches'-broom by cultivation areas and cropping pattern. As a result of examining the disease incidence in the nursery fields for the water dropwort of both Miryang and Uiryong areas in Kyungsangnam-Do, it was accounted for 27% in the Miryang area and 24% in the Uiryong area. In the Miryang area, the disease incidence in paddy fields during 30 days after planting was accounted for 9.71% to 29.93%. Healthy water dropworts and abnormal ones having witches'-broom and tufting were collected and underwent diagnosis by fluorescence microscopy, in the DAPI method, and by transmission electron microscope. As a consequence, specific fluorescence at the phloem elements and mostly pleomorphic phytoplasma in the size of 70-950 nm within tissues unobserved from healthy water dropworts collected in two areas, were observed. Image processing appeared to be effective when diagnose by fluorescence microscopy. To find out the possibility of detecting phytoplasma of infected dropworts by the PCR method, the DNA fraction of the phytoplasma's 16S rRNA gene was amplified by 1.4 kb primers after extracting the entire DNA of both healthy water dropworts and infected ones having witches'-broom or tufting. Specific bands were detected at the position of about 1.4kb on the infected water dropworts of both Miryang and Uiryong areas, but no specific bands were detected from on the healthy water dropworts which were used as control. In water dropwort fields and nursery fields of the Miryang area and their surrounding levees, some insects were examined. Five kinds of Hemoptera, and nine kinds of Homoptera were collected. It was inferred that *Macrostelus striifrons* is vector of the witches'-broom disease of *O. javanica*.

**B-13 Characterization and Functional Analysis of *hpaC* gene carrying Leucine-rich repeat domain in Pathogenicity island of *Xanthomonas oryzae* pv. *oryzae*** Sang Wook Han<sup>2</sup>, Chang-Sik Oh<sup>1</sup>, Byung Kook Hwang<sup>2</sup>, Seung-Joo Go<sup>1</sup>, and Sunggi Heu<sup>1</sup>. <sup>1</sup>Division of Molecular Genetics, Department of Bioresources, NIAST, RDA, Suwon 441-707, Korea. <sup>2</sup> Department of Agricultural Biology, Korea University, Seoul 136-701, Korea

Most plant pathogenic bacteria have host specific interactions with plants. In host plants the bacteria grow to high population levels in the intercellular spaces of the leaf and then produce necrotic lesion. In nonhost plants or in the resistant host plant, the bacteria elicit the hypersensitive response (HR), a rapid, localized defense-associated programmed cell death in contact with the pathogen. These host specific interactions are superimposed on an established basic, species specific parasitism. Genes controlling such fundamental aspects of infection are located on pathogenicity islands (Pai). We have been isolated Pai region from very destructive rice pathogen, *Xanthomonas oryzae* pv. *oryzae*. Up to now we have been sequenced 53 kb DNA region carrying more than 30 open reading frames (ORFs) including genes for type III secretion system. Among those, *hpaC* is encoded by genes located downstream of *hrp* gene cluster. *hpaC* is a 587-amino-acid protein that contains 11 leucine-rich repeat (LRR) domain and possible nuclear localization signal upstream of the leucine-rich repeat. The LRR domain of this protein forms a consensus that perfectly matches the predicted eukaryotic cytoplasmic LRR consensus. For the functional analysis of this gene, transposon mutagenesis and marker exchange mutagenesis had been performed. The function of *hpaC* will be confirmed by the pathogenicity assay. In the further study, we will investigate the possibility that *X. oryzae* pv. *oryzae* delivers HpaC protein to plant cells via the type III secretion system using HpaC-histidin tagging method.

**B-14 Isolation and characterization of genes involved in disease resistance in rice.** Mi-Young Park<sup>1,2</sup>, Hu-Rang Lee<sup>1</sup>, Myung-Ok Byun<sup>1</sup>, Eun-Pyo Moon<sup>2</sup>, and Duk-Ju Hwang<sup>1</sup>. <sup>1</sup>Dept. of molecular genetics, National Institute of Agricultural Science and Technology (NIAST), RDA, Suweon, 440-707 Korea <sup>2</sup>Dept. of Biological Science, Ajou University, Suweon, Korea, 442-749

To investigate disease resistance mechanism in rice, it is necessary to isolate genes involved in disease resistance. We have selected cDNA clones by a reverse northern hybridization using cDNA probes from either BTH or *Xanthomonas oryzae* pv. *oryzae* induced RNAs or untreated RNAs. On the reverse northern hybridization blot, clones showed strong signals in BTH or Xoo treated cDNA probes compared to untreated cDNA probes were selected and sequenced. Based on the NCBI blast program, we have obtained various genes including pathogenesis related protein 1 gene, probenazole inducible protein gene, transcription factors, a gene containing leucine rich repeat. Their expression analysis in different treatment such as pathogens, BTH, and JA will be discussed. Many genes including the PR1 gene were induced in BTH treatment and also upon the pathogen attack.

**B-15 Optimum conditions for antagonistic effect promoting of some antagonistic bacteria.**

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Antagonistic bacteria were isolated from ginseng rhizosphere soils, which were randomly selected at Yuseong and Jinjam area in Taejon. Isolated antagonistic bacteria used in this study were *Erwinia* sp. (S21), *Bacillus* sp. (H106, H077) strains and *Serratia marcescens* (K502, K002), *Pseudomonas fluorescens* (K239, K236, K411) strains donated from Rural Development Administration at Suwon. *Cylindrocarpon destructans*, *Fusarium solani*, *Fusarium oxysporum*, *Botrytis cinerea*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* were used to analyze any effect on pathogenic fungi by the antagonistic bacteria. To select optimum conditions improving antagonistic effect for antagonistic bacteria, these were examined at different media, temperature, and pH conditions. Media were PDA, CDA, LA, V8, and NA, and temperatures selected were 5°C, 15°C, 20°C, and 25°C, and pH were 4, 4.5, 5, 6, and 7. Among eight strains, S21, H106 and H077 strains showed high antagonistic activity and the rests of them were analysed to be not effective. Especially three strains, S21, H106 and H077, showed very effective against growth of *C. destructans*, *F. oxysporum*, and *B. cinerea* with the PDA media at 25°C. Based on these results, this study intended to find a proper promoting condition of the antagonistic activity of the antagonistic bacteria, and to use these conditions for basic information of the biological control to diseases.

**B-16 An Influence of BO Production mutants on the Suppression of Soft Rot and Variation of Pel Activity in *Erwinia carotovora*.** Hyun Jo and Wang Hyu Lee. Department of Agricultural Biology, Chonbuk National University, Chonju 561-756, Korea

*Erwinia carotovora* subsp. *carotovora*(Ecc) has caused soft rot symptom in many plants and produced bacteriocin(BO), antibacterial protein which have antagonistic effect between species. BO was so effect to related strain that it could be limited range. To obtain the non-pathogenic strain from those confirmed strain by BO production, mutagenesis was performed by chemical reagents and then confirmed as a non-pathogenic or weak pathogenic strain. After they were selected, Ecc was inoculated into plant tissue for 3hr pretreatment, post-treatment and simultaneity. However, no symptom was shown in 3hr pretreatment. The degree of soft rot was examined at the ratio of 99:1, 90:10, 50:50, 10:90, 1:99 between pathogen and non-pathogenic mutants. It was significantly decreased in 50:50, 10:90, 1:99 treatments. These results confirmed the decrease effect of soft rot at 1:1 or more ratio between pathogen and non-pathogenic mutants. The Pel activity of non-pathogenic strain was significantly decreased in PYA medium compared to that of parent strain. The result of this experiments indicated that soft rot was suppressed and influenced the variation of Pel activity by BO production of *Erwinia* mutants.



**B-17 Enhancement of Heading date and Yield of Barley and Rice by the treatment with PGPR strains.** Jin Woo Kim, Ok Hee Choi, Sun Mi Lee, Shun Shan Shen, and Chang Seuk Park. Division of Plant Resources and Environment, Gyeongsang National University, Jinju, Korea 660-701.

Significant increases in growth, heading date, and yield of barley and rice were achieved by treating seeds with suspensions of *Pseudomonas* spp. and *Paenibacillus polymyxa* at  $\sim 10^9$  colony-forming units (cfu)/ml prior to planting. The bacterial isolates were selected from over 3000 isolates for *Paenibacillus polymyxa* and 5500 isolates for *Pseudomonas* spp. that were isolated from the root of onion or barley. Bacterization of barley seed planted in field resulted in up to 30% increase in farmer's field. Statically significant increases in yield of rice were 42% in GSNU experimental field. PGPR rhizosphere populations were as great as  $\sim 10^6$  cfu/g of root 4 weeks after plant emergence and averaged  $10^5$  cfu/g for *Pseudomonas* spp. and  $\sim 10^4$  cfu/g for *Paenibacillus polymyxa* throughout the season. The late season plant growth promotion of barley caused by PGPR in different seeding date was followed by enhanced the growth of seedlings, number of tillers, and early heading date compared to untreated controls in GSNU experimental field. Some Pseudomonads enhanced early heading date of rice in GSNU experimental field.

**B-18 Seed priming with PGPR isolates for enhancing germination and early growth of vegetable crops.** Sun Mi Lee, Shun Shan Shen, Hoon Cheong, and Chang Seuk Park. Division of Plant Resources and Environment, Gyeongsang National University, Jinju, Korea 660-701. E-mail changpak@nongae.gsnu.ac.kr

Thirteen isolates were selected among 351 root colonizing isolates for their ability to enhance the roots elongation at early stage of growth. The vegetable seeds, such as pepper, tomato, onion, cucumber, radish, and chinese cabbage were soaked in the cell suspension of the selected PGPR isolates for 1hr and incubate at 28° C for certain period of time then dried in shade and stored. Seeds primed with PGPR isolates showed remarkable enhancement of germination rate and speed. In Petri plate experiments, pepper seeds for instance, 80% of the seeds were germinated within 35hr in *Bacillus* sp. B2-13 treated plot while 64hr in PEG 8000 treated and 78 hr in untreated control. When the Bio-primed seeds were planted in pots, 80% of pepper seeds were germinated within 5 days in B2-13 treated plot, however same rate of germination in untreated plot took 11 days. Seed treated B2-13 colonized on the pepper root system and resulted vigorous and uniform growth of the seedlings

**B-19 Visualizing the infection process of *Xanthomonas campestris* in cabbage using green fluorescent protein.** Hyoung Taek Lim, Hyun Koo Kang, and Jae-Seong So\*. Department of Biological Engineering, Inha University, Incheon, Korea 402-751.

The plant pathogen, *Xanthomonas campestris* NRRL B-1459 was chromosomally tagged with *gfp*, and the transformant which was subjected to Southern hybridization showed the presence of *gfp* in the chromosome. The virulence-related gene of the transformant was not affected by the insertion of *gfp*. After inoculation into cabbage plants, the infection process was visually studied *in planta*. Using a fluorescent microscope, the migration and distribution of *gfp*-labeled bacteria was visualized in real time. As the *gfp*-labeled cells were easily visualized from the beginning of infection, we observed a time delay of 2 days between distribution of the *Xanthomonas* cells in cabbage plant and the appearance of visible necrosis.

**B-20 First Report on Crown Gall of Oriental Melon caused by *Agrobacterium tumefaciens* in Korea.** Young Kee Lee<sup>1</sup>, Dong Geun Kim<sup>2</sup>, Seong Ho Choi<sup>1</sup>, and Jae Soon Cha<sup>3</sup>.  
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Severe incidence of giant gall on lower stem of oriental melon, *Cucumis melo* L., was investigated at Songju, Korea in 2000. Tumors were mostly formed around the grafted area above ground differently from the typical symptom of root-knot nematodes producing galls on the underground roots. The tumors were semi-round with rough surface texture of light brown color and sized 10-20cm in diameter with up to 1.7kg. There was no root-knot nematodes detected from the tumor. Bacterial isolates from the tumor tissue were gram-negative, rod shaped, and produced extracellular polysaccharides on yeast extract-mannitol agar medium. Young tomato plants inoculated with the bacterial developed typical galls within three weeks. Seedlings of oriental melon (cv Geumssaragieuncheon) and root-stock (cv Jangsootozwa) also produced typical galls two weeks after inoculation. The bacterial isolates inducing gall formation in oriental melon were identified as *Agrobacterium tumefaciens* based on biochemical and physiological characteristics, fatty acid profile, and metabolic fingerprints using Biolog GN Microplate. This is the first report on crown gall of oriental melon in Korea.

**B-21 Development of Inoculation Method of Bacterial Canker in Pepper.** Young Kee Lee, Seung Don Lee, and Inn Shik Myung. Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon, Korea 441-707.

This experiment was carried out to develop varietal resistance screening method of pepper against bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis*. Disease severity and symptom development were homogeneous when inoculated by spraying method, whereas there was a significant variation in symptom development when inoculated by soil drenching, injection with syringe or scissor clipping method. Disease severity of pepper plants became severer as age of plant increased from 2-4th leaf stage to 10-13th leaf stage, but there was no significant difference in disease severity between 5-6th leaf stage and older plants. Typical symptoms developed on most leaves and stems at  $10^8$ CFU/ml and there was no difference in disease severity as inoculum density increased from  $10^8$ CFU/ml to  $10^9$ CFU/ml. Disease was the most severe when plants incubated at 25°C after inoculation. Only few number of cankers produced at temperatures lower than 20°C or higher than 30°C. This suggests that inoculation of 5-6th leaf stage plants with  $10^8$ CFU/ml inoculum by spraying method followed by incubating at 25°C was good to assay the virulence of pathogen as well as to assay the resistance of pepper plants.

**B-22 Cloning and characterization of a *popA*-like Gene in the pathogenicity island of *Xanthomonas oryzae* pv. *oryzae*.** Shin Churl Bae, Min-Seon Choi, Chang-Sik Oh, Myong-Ok Byun, and Sunggi Heu Division of Molecular Genetics, Department of Bioresources, NIAST, RDA Suwon 441-707, Korea

The *hrp* genes encode type III secretory pathways and are required by many phytopathogenic bacteria to elicit a hypersensitive response (HR) in nonhost or resistant host plants and for pathogenesis on susceptible hosts. Genes encoding type III secretion systems are present on pathogenicity island (Pai) of plant pathogenic bacteria. Genes encoding effectors such as Harpins of *Pseudomonas syringae* and PopA of *Ralstonia solanacearum* secreted by the type III systems are commonly linked to the type III system genes. We have been isolated Pai region from very destructive rice pathogen, *Xanthomonas oryzae* pv. *oryzae*. Up to now we have been sequenced 53 kb DNA region carrying more than 30 open reading frames (ORFs) including genes for type III secretion system. Among those, a *popA*-like gene, *hpa1* gene encoded a 13 kDa glycine-rich protein with a composition similar to those of Harpins and PopA. Since we believe that the Hpa1 is a kind of a elicitor, we are expressing *hpa1* gene in potato plants under the control of the pathogenic specific promoter *hsr203J*. Also the *hpa1* gene was cloned in pQE-30 vector with the 6×His tag at the N-terminus of the protein and expressed at *E. coli*. Using His antibody, We are investigating whether Hpa1 is secreted into plant cell.

**B-23 Rapid Identification of Plant Pathogenic Enterobacteriaceae Using PCR Ribotyping.** Inn-Shik Myung and Seong-Ho Choi. Division of Plant Pathology, Department of Crop Protection, NIAST, RDA, Suwon 441-707, Korea.

PCR ribotyping of 187 strains of five species of *Brenneria*, two species of *Enterobacter*, six species of *Erwinia*, four species of *Pantoea*, and four species of *Pectobacterium* in plant pathogenic bacterial family Enterobacteriaceae were examined to develop rapid identification technique. Direct PCR from bacterial colony cultured on solid plate with universal primers, 495F and 23r designed from rRNA gene produced two to seven DNA fragments ranged 300bp to 1500bp from all bacteria tested. Although all bacterial species tested grouped to one to four subgroups, each species could be differentiated by size and number of DNA fragments except *Pan. stewartii* and *Pec. carotovorum*. Five subspecies, *atrosepticum*, *betavasculorum*, *carotovorum*, *odoriferus*, and *wasabiae* in *Pectobacterium carotovorum* were classified into four PCR-ribotyping groups, but the PCR-ribotyping group did not accord with the identities of *Pec. carotovorum* subspecies. In addition, strains of two subspecies, *stewartii* and *indologenes* of *Pantoea stewartii* showed the same PCR-ribotyping pattern so that the two subspecies could not be distinguishable by PCR-ribotyping pattern. Results of this study showed that plant pathogenic bacteria in Enterobacteriaceae family could be identified in a level of species by direct PCR ribotyping even on cultured plates.

**B-24 Storage root rot of ginseng (*Panax ginseng* C. A. Meyer) caused by *Paenibacillus polymyxa*.** Yongho Jeon, Young Ho Kim and Ingyu Hwang. School of Agricultural Biotechnology, Seoul National University, Suwon, Korea 441-744.

A bacterial isolate was obtained from a root rot of Korean ginseng (*Panax ginseng* C. A. Meyer) during storage, showing extensive brown rot symptoms. Pathogenicity of this isolate was tested using 4-year-old ginseng roots by wound inoculation or root discs. Conspicuous brown rot symptoms were developed within 3 days at 23°C on ginseng roots which were identical to the original symptoms. The bacterial isolate was recovered from the root rot, confirming the Koch's postulates. The causal organism was identified as *Paenibacillus polymyxa* based on its physiological and biochemical characteristics, and the results of the Biolog program and 16S ribosomal DNA analysis. The isolate also rotted root discs of *Ligusticum acutilobum*, *Codonopsis lanceolata*, *Platycodon grandiflorum* and *Daucus carota* and mushrooms such *Pleurotus ostreatus* and *Lentinus edodes*. To the best of our Knowledge, this bacterium was firstly described as a plant pathogen in the world.