D-01. Isolation, Partial Sequencing and Expression of Pathogenesis-Related cDNA Genes from Pepper Leaves Infected by *Xanthomonas campestris* pv. *vesicatoria*.. Ho Won Jung and Byung Kook Hwang. Department of Agricultural Biology, Korea University, Seoul 136-701, Korea

Specific cDNAs showing differential expression in bacteria-infected pepper leaves as opposed to healthy leaves were isolated from a pepper cDNA library from hypersensitive response (HR) lesions of leaves infected with an avirulent strain of *Xanthomonas campestris* pv. *vesicatoria.* Among a total of 282 cDNA clones tested, 36 individual cDNA genes (13 %) hybridized strongly or differentially to the cDNA probes from bacteria-infected leaves. Ten *Capsicum annuum*-Induced (*CAI*) genes encoding putative thionin, lipid transfer protein I and II, osmotin (PR-5), class I chitinase, β-1,3-glucanase, SAR 8.2, stellacyanin, leucine-rich repeat protein, and auxin-repressed protein were identified. Two *CAI* genes showed little or no sequence homology to the previously sequenced plant genes. Transcripts of the *CAI* genes were strongly or preferentially induced in pepper tissues by infection with *X. campestris* pv. *vesicatoria* or *Phytophthora capsici,* and by abiotic elicitor treatment. In particular, most of the *CAI* genes were strongly induced in pepper tissues by ethephon and methyl jasmonate.

D-02. Pepper Gene Encoding a Novel Thionin is Induced by Pathogens, Ethylene and Methyl Jasmonate. Sung Chul Lee, Jeum Kyu Hong, Young Jin Kim and Byung Kook Hwang. Department of Agricultural Biology, Korea University, Seoul 136-701, Korea

Infection by pathogens or treatment with abiotic elicitors led to a strong expression of thionin gene in pepper (*Capsicum annuum* L.) leaves. The *CATHION1* cDNA which code for thionin were isolated from the cDNA library of pepper leaves infected with *Xanthomonas campestris* pv. *vesicatoria.* Comparison of the *CATHION1* and other known thionin genes revealed that the 8 cysteine residues are at the same location. Modes of transcriptional regulation of *CATHION1* were compared between the compatible and incompatible interactions. Transcripts of the *CATHION1* gene were more induced in the incompatible than compatible interactions of pepper leaves with *X. campestris* pv. *vesicatoria*, but occurred in the contrary manner in the interactions with *Phytophthora capsici.* Transcripts of the *CATHION1* gene were constitutively expressed in healthy pepper stems, but considerably accumulated upon infection by virulent and avirulent isolates of *P. capsici. CATHION1* mRNA was strongly induced by treatment with ethephon or methyl jasmonate, but weakly by salicylic acid and benzothiadiazole. The expression pattern of *CATHION1* gene is time-dependent by treatment with ethephon, but time- and dose-dependent by treatment with methyl jasmonate.

D-03. Soft Rot of Red-pepper Fruit Caused by *Pseudomonas fluorescens* Type II (*P. marginalis*). Hyeok Soo Seo and Young Keun Yi. School of bioresource science, Andong National University, Andong 760-749, Korea

Soft-rot was detected in red-pepper fruits cultivated in the northern Kyeongbuk. The symptom was characterized as the diseased fruits with soft-rotted sarcocarp and decolorized pericarps. Inoculation of the isolated bacterium caused the same symptom on the fruits and the brown necrotic spots on the leaves of the red-pepper plants. This bacterium showed the pathogenicity to twenty different cultivars of red-pepper and on eight kinds of vegetables suseptible to *Pseudomonas marginalis*. The bacterium was identified as *Pseudomonas fluorescens* type II (*P. marginalis*) based on the morphological and physiological characteristics. This is the first report in Korea that soft rot of red-pepper fruit could be caused by *P. marginalis* addition to by *Erwinia carotovora* subsp. *carotovora*.

D-04. Aggressiveness Function of *avrXa7* in *Xanthomonas oryzae* pv. *oryzae*. Seong Ho Choi[1], Jinafa Bai[2], Jan E. Leach[2]. [1]National Institute of Agricultural Science and Technology, RDA. [2]Kansas State University, USA.

Aggressiveness function of the *avrXa7* in *Xanthomonas oryzae* pv. *oryzae* was analyzed by marker exchange mutagenesis of the *avrXa7* locus of Philippine race 2 strain PXO86 followed by complementation of the mutant with functional *avrXa7* gene. *avrXa10* locus also was mutated and complemented with functional *avrXa10* gene to quantitatively compare the aggressiveness function of the two avirulence genes. The two genes have a *Xa7* and *Xa10* resistance gene specific avirulence function, respectively. However, when the *avrXa7* locus was mutated, virulence to susceptible rice cultivar IR24 reduced significantly, while mutation of the *avrXa10* locus did not reduce virulence to IR24. Lesion length on IR24 was 3.4cm 12 days after inoculation with the *avrXa7* mutant, while it was 17.8cm when inoculated with the parental strain PXO86. Number of bacteria in the inoculated leaf of IR24 was 7.6x107 and 3.9x108 10 days after inoculation with the *avrXa7* mutant and parental strain, respectively by leaf clipping method. When IR24 was inoculated with the mutant transformed with plasmid p2A containing functional *avrXa7* gene, lesion length was 13.2cm and bacterial number in a leaf was 1.7x108 that were 74% and 44% of the parental strain PXO86, respectively. Reduction of virulence on IR24 also found in the *avrXa7* and *avrXa10* loci double mutant, which recovered by complementation with functional *avrXa7* but not with *avrXa10* gene. These results indicate that *avrXa7* gene has a dual function such as *Xa7* resistance gene specific avirulence activity and aggressiveness to susceptible rice cutlivar, which suggests that the resistance gene *Xa7* might be durable.

D-05. Bacterial Branch Blight of the Peach Tree(*Prunus persica* var. *vulgaris* Max.) by *Xanthomonas arboricora* pv. *pruni* (Smith) Vauterin *et. al.*

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A new bacterial disease was found on trees of peach(*Prunus persica* var. *vulgaris* Max.) at Kumho, Kyungbuk in April 1999. The disease usually occcurred on over wintered buds, branchs and stems of the Cheon-Hong cultivar. The buds died without sprouting and the branches showed entire wilting. Droplets of bacterial ooze was occasionally running down the surface of diseased plants under moist condition. Artificial needle prick inoculation with isolates obtained from branchs of naturally infected plants produced symptoms similar to those occuring under natural condition.

The causal bacterium was aerobic, Gram nagative, and motile by single polar flagellum. It made yellow colony on beef extract agar. It showed positive reaction in the test of catalase and phenylalanine deaminase, while negative in oxidase, urease and lipase. It produced hydrogen sulfide but did not produced indol. It produced acid from arabinose, dextrin, fructose, mannitol, glucose, glycerol, inositol, maltose, rhamnose, saccharose, soluble starch, treharose and xylose, but not from lactose, esculin, inulin, raffinose and sorbitol.

On the basis of bacteriological characteristics and pathogenicity on the host plant of the organism, the causal bacterium was identified as *Xanthomonas arboricola* pv. *puruni* and this disease was proposed to name "Bacterial branch blight of the peach tree"

D-06. Characterization of a Pathogenicity Island of *Xanthomonas campestris* pv. *glycines*.

Byoung-Keun Park[1] and Ingyu Hwang[2]. [1]Korea Research Institue of Bioscience and Biotechnology, Yusung P.O. Box 115, Taejon 305-600, Korea and [2]School of Applied Biology and Chemistry, College of Agriculture and Life Science, Seoul National University, Suwon 441-744, Korea

We identified a pathogenicity island of *Xanthomonas campestris* pv. *glycines* by mutagenesis with Omegon-Km. Two cosmid clones complementing three nonpathogenic mutants were isolated from the genomic library of *X. campsteris* pv. *glycines*. From DNA sequence analysis of the two cosmid clones, we identified *hrp* gene clusters and other genes important for pathogenicity residing outside of *hrp* clusters. Two cosmid clones were mutagenized with Tn*3gus* to determine roles of each gene in pathogenicity and inducing hypersensitive response on nonhost plants, tomato and pepper plants. Apparently, all *hrp* genes we identified are essential for pathogenicity and inducing HR in pepper. However, contrast to other known *hrp* systems, *hrp* mutants of *X. campestris* pv. *glycines* conferred HR in tomato. We used six different wild types of pepper plants and six different wild types of tomato plants possessing different genetic backgrounds to determine if any genetic variabilities of pepper and tomato plants make any different responses against different mutants. We observed specific interactions between *hrp* mutants of *X. campestris* pv. *glycines* and wild type tomato plants. However, we did not obtain any specific interactions between *hrp* mutants and pepper plants. We will present current status of analyzing a pathogenicity island of *X. campestris* pv. *glycines*.

D-07. Development of PCR Primers for Identification of *Burkholderia glumae*, the Causal Agent of Rice Grain and Seedling Rot. Tae-Whan Nho, Wan-Yeob Song, Mi-Hyung Kang and Hyung-Moo Kim. Department of Agricultral Biology, Chonbuk National University, Chonju, Chonbuk 561-756, Korea

Grain and seedling rot of rice, caused by *Burkholderia glumae*, is a serious emerging seedborne disease of rice. To develop an accurate, rapid PCR-based method for identification, we cloned and sequenced a PCR-amplified fragment containing the Internal Spacer Region between the 16S and 23S rRNA genes from a strain of *Burkholderia glumae*. Primers designated to Ogf4/Ogr3 produced a 402-bp PCR product with each of the 23 strains of *Burkholderia glumae* tested. None of 64 other bacteria tested, including several starins of closely related *Burkholderia* spp., reacted with the primers. Primers Ogf4/Ogr3 are highly unique to *Burkholderia glumae* and should prove to be reliable for rapid PCR-based techniques for identification and detection of this emerging pathogen of rice. Tests are underway to use the primers in a direct-PCR assay using naturally contaminated rice seeds.

D-08. Phylogenetic Analysis of *Pectobacterium* spp. Using the Internal Transcribed Spacer (ITS) Regions and PCR Detection of *Pectobacterium chrysanthemi*. Soon-Wo Kwon[1], Seung-Joo Go, Myeong-Sook Cheon and Jin-Chang Ryu. [1]National Institute of Agricultural Science and Technology, Suwon 441-707, Korea

The phylogentic analyses of *Pectobacterium* spp. were conducted on the basis of ITS region sequences. The *Pectobacterium* species studied contained two types of ITS regions, small ITS and large ITS. The small ITS regions were coding the tRNAGlu and their sizes in *Pectobacterium* spp. were on the range of 354 - 452bp. The large ITS regions contained two tRNA genes, tRNAAla and tRNAIle, and their sizes were 474 - 504bp in length. The sequence homologies of small ITS region among strains of *Pectobacterium* spp. were 42.2 - 93.9%. According to the phylogenetic tree using the small ITS regions, *Pectobacterium* spp. were grouped into two clusters, one cluster including *P. carotovora* subspecies and the other consisting of *P. chrysanthemi* strains. *Erwinia cypripedii, Erwinia amylovora* and *Erwinia pyrifoliae* formed a group clearly separated from two groups of *Pectobacterium* spp. The sequence homologies of large ITS regions among strains of *Pectobacterium* spp. were 45.6 - 95.1%. The phylogenetic analysis on the basis of large ITS region showed the similar pattern with one from small ITS. From the hypervariable regions of *P. chrysanthemi*, a primer set, PCHF and R23-1R, was designed for the PCR detection of *P. chrysanthemi* strains. This primer set was highly specific for *P. chrysanthemi* strains on annealing temperature above 60℃.

D-09. Involvement of Antibacterial Substance Produced by *Pseudomonas fluorescens* in Biocontrol of Bacterial Wilt of Tomato. Jin Woo Kim[1], Byoung Keun Park[2], Ingyu Hwang[3] and Chang Seuk Park[1]. [1]Division of Plant Resources and Environment, Gyeongsang National University # 900 Gazwa-Dong, Chinju 660-701, Korea. E-mail: changpk@nongae.gsnu.ac.kr [2]Plant Protectants R. U., Korea Research Institute of Bioscience and Biotechnology, Yusung P.O. Box 115, Taejon, Korea 305-600. [3]School of Applied Biology and Chemistry, College of Agriculture and Life Science, Seoul National University Suwon 441-744, Korea

Pseudomonas fluorecens B16 has recently been selected as a seed inoculant to vegetable crops to promote seedling growth and increase yields. B16 produces antibacterial substance on minimal media but not on complete media. Of the approximately 5,000 Omegon-Km mutants screened with *Agrobacterium tumafaciens* NTI, we found one mutant (Okm1-36) defective in antibacterial activity and another mutant (Okm2-31) which enhanced its productivity of antibacterial substance. These mutants showed the same effect in other plant pathogenic bacteria, such as *Erwinia carotovora*, *Ralstonia solanacearum*, *Pseudomonas syringae* pv. *syringae*. B16 and Okm 1-36 or Okm2-31 sustained more than 105 cfu per g of root. The population size of Okm1-36 or Okm2-31 was not significantly different from those of wild type strain B16 one month after root inoculation. This indicated that the genes controlling production of antibacterial compound was not closely linked with genes of root colonization. The plants inoculated with the parental strain B16, the mutant Okm1-36, and Okm2-31 are evaluated to find if antibacterial production affects the development of tomato bacterial wilt caused by *R. solanacearum*. The disease incidence of tomato in untreated control and Okm1-36 were 66% and 50%, respectively. But no diseased plant was observed in the plants treated with B16 or Okm2-31. This indicated that antibacterial activity of B16 influences the biocontrol effect of bacterial disease of tomato.

D-10. Genetic Organization and Gene Expression of Bacteriocin Gene Isolated from *Xanthomonas campestris* pv. *glycines* 8ra. Yongsung Kang[1], Moonjae Cho[2] and Yongsup Cho[1] [1]School of Applied Biology and Chemistry, Seoul National University, Suwon, Korea 441-744, [2]Medical school Che-ju National University, Che-ju 690-756, Korea

Antimicrobial activity against close-related microorganisms of plant pathogen *Xanthomonas campestris* pv. *glycines* 8ra is dependent on the production and secretion of a proteinaceous antimicrobial agent called bacteriocin. The DNA region involved in bacteriocin production was localized and its DNA sequences were determined. Based on DNA sequence analysis, two different open reading frames(ORFs) were found and these 2 ORFs are relevant for *in vivo* activity of bacteriocin in *Escherichia. coli*. The 2 ORFs encode each 39kDa and 14kDa polypeptides and they were not significantly related to any other genes. To reveal regulation of bacteriocin synthetic genes and environmental signals involved in the bacteriocin production, a function of promoter was determined by using reporter gene encoding β-glucuronidase. The strain harboring plasmid contained transcriptional fusion of the promoter region of bacteriocin gene and β-glucuronidase gene shows the growth phase-dependent β-glucuronidase gene expression. This tendency of gene expression is often observed in the other bacteria producing bacteriocin.

D-11. Biological Control of Bacterial Leaf Spot on Pepper and Bacterial Leaf Blight on Rice with GlycinecinA Produced by *Xanthomonas campestris* pv. *glycines* 8ra. Yongho Jeon and Yongsup Cho. School of Applied Biology and Chemistry, Colloge of Agriculture and Life Science, Seoul National University, Suwon 441-744, Korea

Xanthomonas campestris pv. *glycines* 8ra produces bacteriocin, glycinecinA, which specifically inhibits the growth of bacteria belonging to *Xanthomonas* spp.. GlycinecinA is very stable at wide range of pH(pH5-pH9) and temperature. Because of these properties, glycinecinA has a possibility as a good biological control agent. We have tested glycinecinA as a biological control agent of *X. campestris* pv. *vesicatoria* and *X. oryzae* pv. *oryzae* in the greenhouse and field for two years. The antibiotic activity is present in cell free culture supernatant of stationary phase culture of *X. campestris* pv. *glycines* 8ra. When the stability of glycinecinA was studied in room temperature and 4℃, it was found to remain constant for up to 2 months in room temperature, and in 4℃, remain constant about 10 months. The minimum activity of glycinecinA to control *X. campestris* pv. *vesicatoria* and *X. oryzae* pv. *oryzae* was 12800Au/ml in greenhouse, and activity of glycinecinA on pepper and rice leaves are each continue for 8 days and 7 days. We have tested the population changes of *X. campestris* pv. *vesicatoria* on pepper leaves and *X. oryzae* pv. *oryzae* on rice leaves. The populations of *X. campestris* pv. *vesicatoria* and *X. oryzae* pv. *oryzae* were monitored for 1 weeks. Compared with treatment with only *X. campestris* pv. *vesicatoria* and *X. oryzae* pv. *oryzae,* the pre-spraying of glycinecinA reduced the populations of *X. campestris* pv. *vesicatoria* and *X. oryzae* pv. *oryzae.* Based on these results, researches of disease control were carried out in greenhouse and field. The bacterial spot disease and bacterial leaf blight each monitered after 4 weeks and 5 weeks from spraying date of *X. campestris* pv. *vesicatoria* and *X. oryzae* pv. *oryzae.* The treatment of spraying with glycinecinA showed significant effect as much as chemical treatment.

D-12. The Bacterial Sheath Rot Disease of Rice Plant by *Burkhorderia glumae*(Kurita & Tabei) Urakami *at. al.* Jin-Woo Lim, Jae-Tak Yoon[1], Jong-Wan Kim College of Natural Resources, Taegu University, Kyungsan, 712-714, Korea, [1]Kyungbuk Provincial Agricultural Technology Administration, Taegu 702-302, Korea

A bacterial disease of rice plant(*Orizae sativa* L.)that rotted the sheath to brown was found in rice plants(Illmi cultivar) at Tanbuk, Uisong, Kyungbuk in June, 1999.

When the bacterial isolates from the diseased rice plants were inoculated by the artificial needle prick method to health plant, the same symptoms were examied.

The causal bacterium was aerobic, Gram negative and motile by 1 to 7 polar flagella. It made fluorescent pigment on potato agar. It showed positive reaction in the test of Kovac's oxidase reaction, arginin dihydrolase, and tween 80 hydrolysis, while negative in M.R.test, V.P.test, levan production, indol production, starch hydrolysis, esculin hydrolysis, production of H2S, phenylanine diaminase, nitrate reduction, potato soft rot, and urease test. It producd acid from arabinose, mannose, fructose, glucose, galactose, glycerol, and xylose, but not from dextrin, inulin, inositol, maltose, lactose, raffinose.

According to its characteristics and pathogenicity on the host plant, the causal bacterium was identified as *Burkhorderia glumae*(Kurita & Tabei) Urakmi *et. al. Burkhorderia glumae* which is known as the pathogen of bacterial grain rot of rice. No report has ever described, however that it causes sheath rot disease of rice plants, It was proposed to call this disease "Bacterial sheath rot of rice"

D-13. Population Genetic Analyses of *Pseudomonas syringae* pv. *actinidiae* Strains from Kiwifruit Orchards. Jae Hong Lee, Jae Goon Lee, Jung Ho Kim, Mi Young Kim and Young Jin Koh. Department of Agricultural Biology, Sunchon National University, Sunchon, Chonnam 540-742, Korea

Genetic relationships of forty-six strains of *Pseudomonas syringae* pv. *actinidiae* and thirty strains of *Pseudomonas* spp. collected from kiwifruit orchards in Korea and Japan were analyzed using the polymorphisms of their indigenous plasmids, chemical resistance and random amplified polymorphic DNAs (RAPDs) of genomic DNAs. Forty strains of *P. syringae* pv. *actinidiae* have one to six plasmids and nine strains of *Pseudomonas* spp. have one to four plasmids, but there was no significant correlation among the types of the plasmids and their origins. Korean strains of *P. syringae* pv. *actinidiae* were relatively more sensitive than Japanese strains to streptomycin, oxytetracycline and copper sulfate and *Pseudomonas* spp. were relatively more resistant than *P. syringae* pv. *actinidiae* to those chemicals. Genetically distinctive subgroups were detected among the populations of *P. syringae* pv. *actinidiae* and *Pseudomonas* spp. by RAPD polymorphisms of genomic DNAs.

D-14. Chemical and Cultural Control of Kiwifruit Bacterial Blossom Rot. Dong Hyun Lee[1], Gyu Hoe Hur[1], Jong Sup Shin[2] and Young Jin Koh[1]. [1]Department of Agricultural Biology, Sunchon National University, Sunchon, Chonnam 540-742, Korea. [2]Yeosusi Agricultural Development and Technology Center, Chonnam 555-130, Korea.

Disease severities of bacterial blossom rot were 13.0~36.2% in naturally infected kiwifruit orchards in Chonnam during 1997~1999 growing seasons. Several control measures were practiced to prevent the occurrence of bacterial blossom rot of kiwifruit in the naturally infected kiwifruit orchards, as compared with chemical spray method. Control effects by spray of Agrimycin (streptomycin sulfate + oxytetracycline wp.) were 65~76% according to the spray times in 1997, whereas those by trunk injection of acetic acids were 73~77% according to the concnetration in 1997 and 1998. Control effects by rainproof cultivation of kiwifruit orchards using vinyl were 61~85% and those by girdling on trunks of kiwifruit trees were 66~69% during 1997~1999, respectively. Trunk injection of acetic acids, girdling on trunks and rainproof cultivation of kiwifruit orchards are considered as reasonable control measures to manage the bacterial blossom rot in kiwifruit orchards.

D-15. Bacterial Soft Rot of *Dendrobium phalaenopsis* and *Phalaenopsis* sp. by *Erwinia chrysanthemi.* Dong Hyun Lee[1], Jung Ho Kim[1], Jae Hong Lee[1], Jae Seoun Hur[2] and Young Jin Koh[1]. [1]Department of Agricultural Biology, Sunchon National University, Sunchon, Chonnam 540-742, Korea. [2]Department of Environmental Education, Sunchon National University, Sunchon, Chonnam 540-742, Korea

Occurrence of soft rots was observed on *Dendrobium phalaenopsis* and *Phalaenopsis* sp. that were grown at greenhouses in Sunchon and Kwangyang, Chonnam. Typical soft rot symptom appeared frequently on young plants of *D. phalaenopsis* and *Phalaenopsis* sp. Symptom began as a small water-soaked lesion on adult leaves of *Phalaenopsis* sp., which enlarged rapidly on the whole leaves and eventually resulted in soft rots of whole plants. Soft rot symptom appeared on adult leaves of *D. phalaenopsis*, and extended into whole leaves, accompanying blighting of whole plants. The causal organism isolated from the infected lesions was identified as *Erwinia chrysanthemi* on the basis of its pathogenicity, physiological and biochemical characteristics, and the results of the Biolog program. The bacterial soft rot by *E. chrysanthemi* was firstly described in *D. phalaenopsis* and *Phalaenopsis* sp. in Korea.

D-16. Phylogenetic Analysis of S-9 Isolate(*Pseudomonas fluorescens*) with Other Pseudomonads by Analysis of 16S rDNA and 16S-23S Spacer Region Sequence. Chul W. Pyun, Seung K. Choi, Ki H. Ryu and Won M. Park. Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea

The isolate S-9, inducing short and thick hypocotyl of soybean sprout, could induce the symptoms on wide range of dicotyledonous plants. The extraction of S-9 cultured medium by ethyl acetate produced similar symptoms on the dicotyledonous seedlings. The pathological studies show that this S-9 isolate is a new strain of *P. fluorescens* and could be clearly distingushed from other fluorescent Pseudomonads. Recent advances in molecular ecological techniques and taxonomy open ways to design highly specific PCR protocols, especially for detection of 16S rDNA genes and 16S-23S spacer region.

Two sets of primers were designed from the sequence of the 16S rDNA gene of the *Pseudomonas* sp. They were a forward 18-mer primer, 5 CGGATTAGCTAGTTGGTG 3 (PSUP; GenBank accession No. AJ 011353) and a reverse 18-mer primer, 5 GCGATTACTAGCGATTCC 3 (PSDN; GenBank accession No. AJ 011354). The S-9 isolate 16S rDNA fragments were obtained by amplification in PCR with the primers. PCR amplification of the 16S-23S spacer region was performed by using the PCR primers that we designed by inspecting the sequences of 16S rRNA and 23S rRNA genes conserved in various *Pseudomonas* sp. Two sets of primers were a forward 21-mer primer, 5 CGGCTGGATCACCTCCTTA AT 3(S-9UP) and a reverse 23-mer primer, 5 CTTCTTCACTTGACCATATAACC 3(S-9DN). The 16S rDNA fragments and the S-9 isolate 16S-23S spacer region fragments were cloned into *p*CR 2.1 vector (Invitrogen) and *p*GEM-T Easy vector (Promega). Recombinants plasmids were transformed to *Escherichia coli* T-9. Nucleotide sequence was analyzed in both directions by the dideoxynucleotide chain termination method using the Model 377 DNA Sequencer (ABI). Nucleotide sequences were analyzed by DNASTAR software package (Madison, WI).

The phylogenetic analysis showed that the S-9 isolate 16S rDNA can be used to differentiate closely related strains within *Pseudomonas* species rRNA homology group I of GenBank database. The S-9 isolate 16S-23S spacer region showed about 70% homology with *P. syringae* of GenBank database.

D-17. Genetic Analysis of *Xanthomonas axonopodis* pv. *citri* and *X. a.* pv. *aurantifolii* Using Random Amplified Polymorphic DNA Fragments (RAPDs). Inn-Shik Myung. Agricultural Science and Technology, RDA, Suwon, Korea 441-707. [2]School of Applied Biology and Chemistry, Seoul National University, Suwon, Korea 441-744. [3]Fruit Laboratory, USDA/ARS, BARC West, Beltsville, MD 20705, USA

Genetic diversity among 57 strains of *Xantomons axonopodis* pv. *citri* and 18 strains of *X. a.* pv. *aurantifolii*, associated with different forms of citrus bacterial canker disease was investigated using Random Amplified Polymorphic DNA (RAPD). One hundred and seventy-five different RAPD fragments were consistently produced by polymerase chain reaction (PCR) amplification with 19 different 10-mer primers. Genetic distances among the bacterial strains were calculated by Nei's algorithm and phenetic tree was derived from the Unweighted Pair Group Method with Arithmetic Mean Analysis (UPGAM). Three different patterns, A - B/C/D, A/B/C/D, and A - B/D - C were observed in RAPD profiles, depending on primer used for PCR amplification. The cluster anlaysis of RAPDs data revealed phenetic clusters similar to those obtained previously from RFLP analyses of genomic DNA and plasmid DNA fingerprintings. Dendrogram derived from combined RAPDs data showed that the two pathovars were clearly differentiated at a level of about 24 % dissimilarity. *X. a.* pv. *citri* strains were further separated into two groups. Forty-nine strains of *X. a.* pv. *citri* formed a single group, whereas the other strains that included 5 ones previously reported as variants did a second group. Strains of *X. a.* pv. *aurantifolii* were also separated into two distinct groups; one was with pathotype B/D and the other one was with only pathotype C. This study shows that strains of *X. a.* pv. *citri* and *X. a.* pv. *aurantifolii* exhibit genetic diversity detectable by RAPD analysis, and that cluster analysis of RAPD fragments can be used both to distinguish between strains of each pathovar and to determine relatedness between them.

D-18. The Influence of Bordeaux Mixture Spraying After Peach Harvest on the Occurrence of Bacterial Shot Hole. San-Yeong Kim, Tae-Young Kwon, Im-Soo Kim. Cheongdo peach Experiment Station, Kyongbuk Province ATA, Cheongdo 714-850, Korea

This experiment was carried out to investigate the effect on prevention of bacterial shot hole, when Bordeaux mixture is sprinkled on peach trees after peach-harvest.

We sprayed Bordeaux mixture on 'Mibaekdo' peach trees 1 to 3 times after mid-September, and then next year examined how much bacterial shot hole occurred. Bacterial shot hole in leaves appeared from mid-May and thereafter increased continuously. The more we sprayed Bordeaux mixture, the less peaches were attacked with bacterial shot hole. At the beginning of August, the peach harvest time, the rate of leaf attack of untreated control plot was 27.4%, on the other hand, when Bordeaux mixture was sprayed, the rate of it was 14.9%, 11.8%, 9.7%. The control value ranged from 45.6% to 64.6%.

The occurrence of fruits is similar to that of bacterial shot hole in leaves. When no treatment was given, rate of fruit attack was 19.4%, but in case of the opposite was 11.1%, 9.5%, 6.4%. This shows 42.8%~67.0% in the control value. Sprinkling of Bordeaux mixture from mid-September to mid-October didn't do peach trees any chemical injure. In terms of economic analysis, the index of income is 110.

D-19. PCR DNA Fingerprints of Phytopathogenic Fungi and Bacteria Using URP Primers Designed from Rice Repetitive Sequence. Hee-Wan Kang[1], Seung-Joo Go[1], Young-Jin Park[1], Seung-Hun Yu[2], Hong Ki Kim[2] and Jin-Chang Ryu[1]. [1]Division of Molecular Genetics, National Institute of Agri. Science & Technology, Suwon 441-707, Korea, [2]2Department of Agricultural Biology, College of Agriculture, Chungnam National University, Taejon 305-764, Korea

URP (Universal Rice Primer) primers designed from repetitive sequence of rice have universally been used in evaluating DNA diversity of various organisms including plants, animals and microorganisms. In this study, the URP primers were applied for identifications between and within species of phytopathogens including fungi and bacteria. PCR reaction using each single URP primer of 20 mer was performed on a highly stringent condition with annealing temperature of more than 55℃ to improve PCR reproducibility, which was problematic in RAPD. Six URP primers could reproducibly produce PCR DNA fingerprints at levels of species or strains of a variety of fungi, including *Alternaria* spp, *Penicillium* spp, *Fusarium* spp. and bacteria with *Erwinia* spp., *Pseudomonas* spp., *E. coli,* and *salmonella* spp. Furthermore, URP-PCR products sharing in *Alternaria kikuchiana* and *Erwinia carotovora* subsp. *carotovora* could possibly be used to generate specific DNA markers for detecting the pathogens.

D-20. Strategy for Maximizing Expression and Single-Step Purification of a *Chromobacterium* sp. Strain C-61 Chitinase in *Escherichia coli.* Seur Kee Park[1], Young Cheol Lee[1] and Jae Sung Jung[2]. [1]Department of Applied Biology and Horticulture, [2]Department of Biology, Sunchon National University, Sunchon, Chonnam 540-745, Korea

Escherichia coli harboring a chitinase gene (*ChiCV*) of *Chromobacterium* sp. formed clear zone on the agar plate containing colloidal chitin when the gene was constructed in pBK-CMV vector. Extracellular production of the chitinase was highest when cultured for 16 hours in Luria-Bertani Medium (LB) and for 5 days in 1/2 LB containing colloidal chitin. In the optimal culture period, the chitinase activity was much higher in 1/2 LB containing colloidal chitin than in LB. The *ChiCV* was constructed in pET-36 vector that has a CBD-Tag cellulose binding domain and a S-Tag peptide for affinity purification. *E. coli* harboring the recombinant plasmid did not form clear zone on the agar plate containing colloidal chitin and its chitinase activity was slightly detected in the culture supernatant. Thus, fusion proteins with chitinase activity were isolated and purified from cytoplasmic proteins and periplasmic proteins of recombinant *E. coli* cells. Production of the fusion proteins increased by addition of IPTG and was more superior in Terrific Broth (TB) than in LB. When the proteins were passed through CBD-Tag and S-Tag column, purity of the fusion proteins was more superior in the periplasmic proteins than in the cytoplasmic proteins.

D-21. The Roles of Domains of a Chitinase from *Chromobacterium* sp. Strain C-61 in Chitin Degradation. Seur Kee Park[1], Jae Sung Jung[2] and Hoon Kim[3]. [1]Dept of Agricultural Biology, [2]Dept of Biology, [3]Dept of Agricultural Chemistry, Suncheon National University, Chonnam 540-745, Korea

The chitinase from *Chromobacterium* sp. C-61 consisted of a signal peptide of 26 amino acids and a mature protein of 510 amino acids. The deduced mature protein contained three functional domains such as a chitin-binding domain (CBD) in N-terminal, a bacterial extracellular solute-binding protein (BBP) in the middle region and a catalytic domain in C-terminal. The catalytic domain had three chitinase active sites (CAS I, II and III) that were belong to family 18 of glycosayl hydrolases. To define the roles of these domains in chitin degradation, domains of the chitinase gene (*ChiCV*) was deleted by deletion- and site-directed mutagenesis. *Eschericha coli* harboring the intact *ChiCV* gene formed clear zone on the chitin agar plates and secreted the chitinase into culture supernatant, which had molecular weight of 54- and 53-kDa on SDS-PAGE. However, Deletions of signal sequence from ChiCV did not secrete the chitinase into outside of *E. coli* cell wall, although chitinase of 54- and 53-kDa was produced in the cytoplasm. While, deletions of BBP, and CAS I, II and III from *ChiCV* did not have a chitinase activity. Deletion of CBD were lower in ability to degrade colloidal chitin on the agar plate, in chitinase activity toward native or colloidal chitin, although not differed in chitinase activity against *p*-nitrophenyl-b-D-*N,N*-diacetylchitobiose (*p*NP-(GlcNAc)2

D-22. The Promoting Effect on Viability in Ginseng Rhizosphere Soil by Formulation of Antagonistic Bacteria. Sung Joon Yoo[1], Sun Ick Kim[2], Jung Ju Lee[1] and Hong Gi Kim[1]. [1]Department of Agricultural Biology, Chungnam National University, Taejon, Korea 305-764. [2]Agriculture Technology and Extension Center, Kumsan gun 312-835, Korea

Antagonistic rhizobacteria *Erwinia* sp. S21 for biocontrol against soil born fungal disease of ginseng was treated to soil substrates and ginseng seedling against multiple ginseng pathogens. To improve viability of S21 in a ginseng field, various formulations were tested. Dynamic activities of S21 in soil substrates were studied with various bacteria population which were isolated using isolation techniques and based on existence of the plant growth promoting effect and multiple ginseng pathogens attack depressing action. Formulations used in this study were 1) powder formulation which were the most effective for the ginseng seedlings and 2) soil amendment which was mixed of 1,000kg of rice husk, 150kg of rice bran and 50 ℓ of antagonistic bacteria (S21)(5×108 cfu/㎖) applied to ginseng field soil. Four types of field treatments; 1) powder formulation on seedlings and amendment on soil, 2) powder formulation on seedlings without treatment on soil, 3) soil amendment on soil without treatment on seedlings, and 4) no treatment on both seedlings and soil were studied in this study. Isolation of S21 was conducted after 100 days once ginseng seedlings were planted. The highest isolation rate of S21 obtained from the treatment which was 2.6× 106cfu/g soil. Isolation of S21 treatment 1) and 3) was 0.5×106cfu/g soil and 0.5×106cfu/g soil, respectively. No isolation was obtained from treatment 4) Antagonistic effect of reisolated S21 tended to keep and found against *Fusarium solani*. After dilution plating, the population ratio of S21 from total bacteria in rhizosphere soil was 0.6, 0.4, and 2.3% in treatment of 1), 2) and 3), respectively. The rate of diseased plant was much higher on no treatment than treatment after 100 days. There was, however, no significant difference on plant height and leaf width among treatments.

D-23. Treatment of Formulated Antagonistic Rhizobacteria for Biocontrol of Root Rot Disease of Ginseng in Pot. Sung Joon Yoo[1], Sun Ick Kim[2], Jang Ho Lee[3] and Hong Gi Kim[1]. [1]Department of Agricultural Biology, Chungnam National University, Taejon, Korea 305-764. [2]Agriculture Technology and Extension Center, kumsan gun, Korea 312-835. [3]Korea Ginseng & Tobacco Research Institute, Taejon 305-345, Korea

Antagonistic bacteria *Erwinia* sp., which has biocontrol effect against soil-borne ginseng fungal diseases, was applied to soil substrates in pot to study disease control effect. Contagious soil was obtained from the korean tobacco and ginseng research institute and diluted to 50%. The size of pot was $\phi 40 \times h$ 50㎝ and five ginseng seedlings were planted per pot. Three different treatments, 1) S21 treatment, 2) magnesium treatment which was known for biocontrol effect among farmers, and 3) no treatment were carried out. The population of S21 in power formulation resulted from dilution plating method was 5×108 cfu/g. The rate of diseased plant after 100days treatment in treatment 1), 2) and 3) was 13, 53%, and 86%, respectively. The S21 showed the high antagonistic effect against ginseng pathogens. And also, S21 highly reisolated about 4×108 cfu/root from ginseng seedling surface after 100days treatment.

D-24. Comparative Analysis of Active Oxygen Species(AOS) Signal Pattern of Host HR and Nonhost HR in Red Pepper. Youn Sig Kwak, Dowg won Bae, Byung Soo Kim[1], Hee Kyu Kim. Dept. of Agricultural Biology, Gyeongsang National University [1]Dept. of Horiculture, Gyeongpook National University

We have assayed the generation of active oxygen and it's scavenging enzyme activity during the nonhost HR(pepper-*Xanthomonas campestris* pv. *campestris*) and host HR(pepper-*Xanthomonas campestris* pv. *vasicatoria* Race 1). For nonhost HR, EPR signal increased in 30min, 6hr and 24hr. Correspondingly, isozyme of SOD was detected in 24hr. H2O2 accumulated distinctly in 30min(phase Ⅰ) and 3hr(phase Ⅱ). CAT activity was increased in 15min and decreased in 6hr and isozyme of POD appeared in 3hr. Whereas for host HR, EPR signal was increased in 24hr and H2O2 accumulated in 3hr and 24hr. Nitric Oxide accumulated drastically in early stages within 1hr after inoculation for both nonhost and host HR, which lasted in similar pattern for 6hr. LOX activity in 30min and 1hr higher for host HR than for nonhost HR. But in later stage after 6hr, high enzyme activity was maintained for both HR reaction. DAPI stain revealed DNA chromatin concentration for both host HR and nonhost HR. H2O2 treatment as a trigger for HR did not resulted in cell death. SNP, trigger for NO production, induced cell death at concentration of 3mM. SA and LA treatment also reacted in cell death at concentration of 5mM and 10mM, respectively. Above four triggers, if combined at sublethal concentration, induced cell death in vivo. Inhibitors, DPI(1mM), PTIO(1mM), DMTU(40mM), Qucertein(5mM), treated at given concentration were not sufficient enough to protect the treated leaves from cell death. Protein two dimensional electrophoresis revealed 24 spots in nonhost HR and 22 in host HR. We also observed 16 by SA, 11 by LA, 35 spot by SNP treatment.

D-25. A Semiselective Medium for the Detection of Soybean Sprout Soft-Rot Causing Bacteria from Soybean Seeds. Su-Hyun Kim, Wan Yeob Song, Mi Hyung Kang and Hyung Moo Kim. Agricultral Biology, Chonbuk National University, Chonju, Chonbuk 561-756, Korea

A semiselective agar medium, designated to mannitol- asparagine-tween 80 (MAT) medium, was developed for the efficient detection of bacterial soybean sprout soft-rot pathogen, *Erwinia carotovora* subsp. *carotovora*, from soybean seeds. The selective medium contained 10.0 g mannitol, 3.0 g L-asparagine, 2.5 g taurocholic acid, 10.0 ml tween 80, 17.0 g agar, 10.0 mg bromphenol blue and 2.5 mg tetracycline per a liter. Pin-point showing lipid-hydrolysis zone was appeared after the inoculation of soft-rot bacteria by spetulating diluted cell suspension and incubating for 12 hr on the MAT medium. Colonies of pathogen were 2.0 - 2.5 mm in diameter, circular, pulvinate, entire and light blue after 3-day incubation at 28 ℃. Lipid hydrolysis zone around the colony on MAT medium made easy differentiation of the casual pathogen from other seed borne bacteria of soybean seeds.

12hr-shaking incubation at 4 ℃ in 0.85% saline contained 0.01% tween 80 was the optimal extraction condition for extracting the cells of soft-rot bacteria. Mean recovery percentage from 9 strains of the bacterial soft-rot pathogen was 102.0% and from mixtures of inoculum and soybean seeds was 81.3% on MAT medium in comparison to King's medium B as a general medium.