B-20. Biocontrol to root rot disease and growth promoting effect of ginseng by formulation with *Burkholderia pyrrocinia* and *Bacillus subtilis*. Jae Beom Ra¹, Chang Soon Jang¹, Doo Won Kwak², Sung Joon Yoo², and Hong Gi Kim¹.

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Effect of the biocontrol against Cylindrocarpon destructans and the increased growth promotion in ginseng by formulations with Burkholderia pyrrocinia and Bacillus subtilis demonstrated as effective biocontrol agents were investigated in continuous cropping ginseng field and soil fumigated ginseng field. Product formulations (A; sawdust type, WP; wettable powder type, C; zeolite type) were tested to develop a biocontrol agent containing both B. pyrrocinia and B. subtilis. Three treatments were conducted: 1) four formulations (C2, G2, WP2, Z2) were treated to seedlings when transplanted, 2) four formulations (C3, G3, WP4, Z3) were treated to soil before seedlings were transplanted and were treated to seedlings when transplanted, and 3) dipping (WP3) was treated to seedlings when transplanted. For the first three month, the highest germination rate of 87.7% and 98% was shown with the formulation WP3 (wettable powder type) in continuous cropping and soil fumigated ginseng field, respectively. In continuous cropping and soil fumigated ginseng field, the disease index of ginseng after five months treatment was 7.7% and 12.5% (G3), 11.4% and 15.9% (WP3), 33.3% and 48.9% (Control), respectively. Furthermore, highly growth promoting rate was 63.5% and 40.7% (WP3), 31.5% and 23.3% (G3), respectively. Initial result obtained from those field trials implied the biocontrol agent in formulation WP and G was very promising to protect ginseng from the root rot pathogen, C. destructans and to have highly growth promoting effect of ginseng.

B-21. Field tests of antagonistic bacteria for biological control of phytophthora blight of pepper. Hye Sook Kim and Ki Deok Kim. Division of Bioscience and Technology, Korea University, Seoul 136-701, Korea.

In our previous studies (Plant Pathol. J. 17:371), four root-associated bacteria were selected through radicle and seedling assays including plant tests using 231 bacterial strains. In this study, using the selected strains with metalaxyl, field tests with artificial inoculation were conducted at Deokso Research Farm of Korea University, Namyangju, Korea in 2001 and 2002. In 2001 test, three potential antagonistic strains, KJ1R5, KJ2C12, and KJ9C8 produced significantly (*P*=0.05) lower disease severity and incidence than MgSO4 buffer-treated controls since 18 days after inoculation (DAI). However, the application of strain 11S16 failed to reduce Phytophthora blight of the pepper in the field. Metalaxyl induced the lowest disease severity and incidence among the treatments. In 2002 test, two strains, KJ1R5 and KJ2C12 produced significantly (P=0.05) lower disease severity than the buffer-treated controls since 8 DAI while did lower disease incidence during 8-14 DAI. However, strains 11S16 and KJ9C8 generally did not reduce the disease, but metalaxyl did the lowest among treatments. Analysis of AUDPC also showed similar results both in 2001 and 2002. Therefore, results from field tests have proven that our previous radicle and seedling assays were very effective,

useful for pre-selecting antagonistic bacteria for biological control of Phytophthora blight of pepper.

B-22. Suppression of cucumber damping-off, caused by *Rhizoctonia solani*, by *Burkholderia* spp. *Y.-S. Bae*, T.-Y. Yun, D.-G. Kim, K.-S. Park, S.-B. Lee, and C.-H. Kim Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon, 441-707, Korea.

Pre- and post-emergence seedling damping-off of many crops caused by *Rhizoctonia solani* are one of important diseases in Korea. From 2001 to 2002, more than 1100 bacterial isolates were collected from the rhizosphere of many crops grown in fields. Among them, several *Burkholderia* species were selected as a promising biocontrol agent through four times greenhouse bioassay conducted in soil artificially infested with *R. solani*. Cucumber seed priming with cell suspension of bacterial isolates resulted in successful suppression of cucumber damping-off caused by *R. solani*. This paper presents their ability to control damping-off in soils containing different levels of pathogens inoculum and characteristics related to biocontrol ability.

B-23. Protection of tomato against Fusarium crown and root rot by seedling treatment with endophytic bacteria in greenhouse. J. T. Kim¹, K. Y. Ryu¹, J. S. Kim¹, and S. H. Yu². ¹Highland Crop Research Divison, National Highland Agricultural Experiment Station, RDA, Pyungchang, Gangwon 232-950, Korea, ²Department of Agricultural Biology, Chungnam National University, Daejon 305-764, Korea.

Eight endophytic bacteria were obtained from internal tissues of tomato for biocontrol against crown and root rot of tomato caused by *Fusarium oxysporum* f. sp. radicis-lycopersici (FORL). By root-dip method, these strains were bacterized with tomato seedlings. The disease severity was evaluated at 30 days after transplanting. The bacterized tomato seedlings grew vigorously and they also reduced of disease incidence compared with that of non-treated plant. The four most suppressive strains were examined further for their tomato yields and suppression of disease severity in infested-soil with FORL pathogens. These strains were PYT-40, PYT-86, PYT-87, and GST-188, which were identified as *Alcligenes* sp., *Stenotrphomonas* sp., *Bacillus* sp., and *Pseudomonas* sp., respectively. Two strains, PYT-40 and GST-188, significantly reduced the incidence of disease severity (disease index 1.6 to 1.8 versus control 2.3), and tomato yields by the treated plants increased as compared with non-bacterized control plants under greenhouse condition for four months.

B-24. Selection of endophytic bacteria and their antagonists to Fusarium dry rot of potatoes. J. T. Kim¹, J. S. Kim¹, K. Y. Ryu¹, J. U. Cheon¹, and S. H. Yu². Highland Crop Research Division, National Highland Agricultural Experiment Station, RDA, Pyungchang, Gangwon 232-950, Korea, ²Department of Agricultural Biology, Chungnam National University, Daejon 305-764, Korea.

One hundred seventy-six endophytic bacteria from potato (Solanum tuberosum L.) and tomato (Lycopersicon esculentum M.) plants were tested in vitro bioassays using tuber disk techniques for suppressiveness to Fusarium dry rot of potato. In Gangwon province, several species of Fusarium oxysporum, F. solani, F. sambucinum and F. equiseti were identified as the major pathogens causing dry rot of storing potatoes. All strains were individually assayed by coinoculating bacterial strain and pathogens on cv. Superior tuber disk. Six bacterial strains consistently suppressed dry rot incited by four different species of Fusarium. Inhibitory activity of four bacterial PEB-63, PEB-78 and TEB-40) was significantly (P<0.01) against F. oxysporum, F. sambucinum and F. equiseti than F. solani. However, two of six bacterial strains, PEP-112 and PEP-114 were suppressive against four species of Fusarium. These antagonistic strains included members of the genera Bacillus, Pseudomonsa, Enterobacter, and Alcaligenes.

B-25. Biocontrol of chestnut blight cankers by using hypovirulence. Sang-Hyun Lee¹, Jong Kyu Lee², Kyung Hee Kim¹, and Byung-Ju Moon³. ¹Department of Forest Diseases and Insect Pests, Korea Forest Research Institute, ²Division of Forest Resources, Kangwon National University, ³Faculty of Natural Resources and Life Sciences, Dong-A University

Ten virulent(V) strains of Cryphonectria parasitica, which are representative of each vegetative compatibility(vc) groups, were converted to hypovirulent(H) strains through pairing and hyphal anastomosis of a H strain, KCPH-22. Most of the pairings produced barrage lines on culture medium, but a V strain, KCPV-19, was successfully converted to hypovirulent. Cultural characteristics of the converted hypovirulent(CH) strain was changed to those similar to the original H strain used in conversion. Mycelial growth rate was noticeably decreased, and colony was whitish in color. Converted strain also contained dsRNA virus originated from the H strain(KCPH-22). Biocontrol efficacy of chestnut blight cankers was evaluated by using both V and H strains, which were successfully paired strains in hypovirulent conversion, on living chestnut seedlings. The H strain(KCPH-22) was treated on the margins of canker, which was developed in 4 weeks after inoculation with the V strain(KCPV-19). Callus tissue was developed on the bark tissue at the margin of canker by the treatment of KCPH-22, and the expansion of canker development was finally stopped. Re-isolation and culturing of chestnut blight fungus was done from the canker area. It was confirmed that the V strain was converted to the H strain.

B-26. Weeding efficacy of phytotoxin extracted from *Sclerotinia* sp. (BWC98-105) on the white clover *Trifolium repens*. Y. K. Hong, J. N. Hyun, S. B. Song, B. C. Lee, D. C. Lee, and S. C. Kim. National Yeongnam Agricultural Experiment Station, Milyang 627-803, Korea

Sclerotinia sp. (Isolate BWC98-105) causes stem blight, root rot in Leghum sp. and is presently being evaluated by author as potential mycoherbicide for the control of white clover(Trifolium repens). Bioassays with liquid cultured mycelia had shown that the Sclerotinia produces phytotoxins biologically active against T. repens. Two

biologically active compound, Designated toxin I and toxin II were produced from culture filtrate of *Sclerotinia* isolate BWC98-105 *in vitro*. The toxin I and toxin II were purified by means of liquid-liquid extraction and C18 open column chromatography(300 ×30 mm, i.d). To determin the purity, the purified toxin I and toxin II were analyzed by RP-HPLC. The flow rate was set at 0.7 ml/min. using linear gradient solvent system initiated with 15 % methanol to 85 % methanol in 50 min. with monitoring at 254 nm. Under these RP-HPLC conditions, the toxin I and toxin II were eluted at 3.49 and 4.13 min. respectively. Toxin II shown to be most potent and host specific to white clover. No toxin activity was detected in the water fraction after partitioning with several organic solvent but only in butanol phase. In the leaf bioassay using toxin II with the contration of 10 μ g a.i, first appeared within 1 hr as a water soaked which subsequently developed into well-defined blighted leaves. After all, the roots of the white clover were blighted as well as the whole aerial part of the plant. So, we concluded that the toxin II purified from culture extract of Sclerotinia sp. is a potential compound for control agent of *T. repens*.

B-27. A phosphate-solubilizing bacterium, *Enterobacter intermedium* 60-2G, induces plant disease resistance and promotes plant growth. C. H. Kim, W. J. Ha, K. Y. Kim, B. H. Cho, and Y. C. Kim. Agriculture Plant Stress Research Center(APSRC), College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Korea

Enterobacter intermedium 60-2G, was examined for its plant growth-promotion activity in cucumber and for inducing activity of systemic resistance of cucumber to scab pathogen, Cladosporium cucumerinum. Compared with a nonbacterized control, root colonization of E. intermedium significantly reduced the severity of scab disease caused by Cladosporium cucumerium. Treatment of cucumber with E. intermedium also promoted cucumber growth. The 60-2G strain showed a strong antimicrobial activity against several plant pathogenic fungi including Fusarium oxysporum and Magnaporthe grisea in vitro. These results suggest that E. intermedium 60-2G is a promising candidate as a biological control agent displaying multiple beneficial properties to promote plant health. E. intermedium 60-2G possessing a strong ability to solublize in soluble phosphate was isolated from rhizosphere of grass. Phosphate solublizing ability of E. intermedium 60-2G is accomplished by production of gluconic acid through a direct extracellar oxidation of glucose by glucose dehydrogenase that required a cofactor, pyrroquinoline quinone(PQQ), for its activation.

B-28. Three bacterial metabolites of *Burkholderia gladioli* IN26 related to induce systemic resistance and antibiosis against fungal plant pathogens. <u>K.-S. Park¹</u>, E.-Y. Kim¹, M.-S. Kwack¹, Y.-S. Bae¹, S.-B. Lee¹, C.-H. Kim¹, and J. W. Kloepper². ¹Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707 Korea, ²Department of Entomology and Plant Pathology, Auburn University, Auburn AL 36849, USA

Burkholderia gladioli strain IN26, which was isolated from the rhizosphere of cotton, induced systemic resistance against cucumber anthracnose, bactertial leaf spot of

cucumber, and wild fire of tobacco. The isolate elicits both PR-a promoter expression and HR reaction as well as antifungal activity against major fungal plant pathogens. To identify the bacterial determinants involved in induced systemic resistance, a water soluble cell wall component was isolated from IN26 grown on King's B agar and purified using various columns including charcoal fractionation. The partially purified extracellar polysaccharide induced systemic resistance in cucumber against cucumber anthracnose and powdery mildew when pre-infiltrated at 200 ppm. Two unknown compounds were also purified from the culture filtrate of IN26 using silicagel G, C-18, and LH-20 column chromatography. One of the compounds completely inhibited the growth of *Rhizoctonia solani* and *Colletotrichum orbiculare* at 10 ppm. The other compound induced the expression of PR-1a encoding GUS reporter system, indicating one of bacterial determinants involved in induced systemic resistance. These results suggest that a PGPR strain IN26 simultaneously produces several determinants related to ISR or antibiosis.

B-29. Induction of systemic resistance in cucumber against *Colletotrichum orbiculare* by cyclic dipeptide, Cyclo (L-Pro-L-Tyr) purified from plant growth promoting rhizobacterium, *Bacillus amyloliquefaciens strain* EXTN-1. K.-S. Park¹, E.-Y. Kim¹, M.-S. Kwack¹, S.-C. Cho¹, S.-S. Moon², and C.-H. Kim¹. ¹Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707 Korea, ²Department of Chemistry, Kongju National University, Kongju, Korea

Selected strain of the plant growth promoting rhizobacterium, Bacillus amyloliquefaciens EXTN-1, has been reported to induce multiple-spectrum systemic resistance in several crops as well as plant growth promotion. In previous paper, we reported that the plant showed oxidative burst, lignification, and expression of pathogenesis-related genes when cell suspension of EXTN-1 was treated to cucumber This paper reports the possible defense mechanism associated EXTN-1-mediated ISR. Treatment of EXTN-1 on Arabidopsis wild type Col-0 resulted in the activation of PR-1 and PDF 1.2 genes simultaneously. Cyclic dipeptide, cyclo (L-Pro-L-Tyr), was isolated by various column chromatography from culture filtrates of EXTN-1. The compound was identified as cyclo (L-Pro-L-Tyr) based on analysis of NMR spectra. This compound induced systemic resistance in cucumber plant agianst Colletotrichum orbiculare. Infiltration of this compound at the low concentration (0.001 ug/ml) induced the expression of PR-1 promoter, encoding GUS activity. These results suggest that a bacterial metabolite, cyclo (L-Pro-L-Tyr) involves in the activation of plant defense reactions, leading to systemic resistance against cucumber anthracnose fungi.

B-30. Involvement of heat-stable and protenaceous material in the culture of *Psedomonas putida* JB-1 for the inhibition of tobacco mosaic virus Infection. Yong Ho Jeon and Young Ho Kim. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

A variety of fungi and bacteria were tested for their antiviral activity against

Tabacco mosaic virus (TMV) infection on a local lesion host, Nicotiana tabacum cv. Xanthi-nc NN. Among 5 bacterial isolates showing antiviral effects, one isolate named JB-1 was most active, which was identified as Pseudomonas putida based on physiological, Biolog and partial 16S rDNA sequencing analyses. Simultaneous treatment of the bacterial culture inhibited almost completely the TMV infection consistently, and 80% inhibition was obtained 12 hours after treatment. When the bacterial culture was applied to the abaxial leaf surface, TMV infection to the abaxial leaf surface was inhibited by about 50 %, showing a local systemic effect. Also treatment of the culture to plant rhizosphere induced a resistance against TMV infection (induced systemic resistance). Active components for the antiviral activity were hydrophilic, and were produced most by culturing the bacterium for 4 or more days. TMV infection was inhibited as effectively by the heat-treated and even boiled bacterial cultures as the untreated control one. However, the treatment of proteinase K nullified the antiviral activity of the bacterial culture almost completely. These results suggest that the antiviral substance(s) may be protenaceous in nature and very heat-stable.

B-31. Characterization and biological properties of *Streptomyces kasugaensis* GBA0927 production. <u>Hyang-bok Lee</u>, Ji-Tae Kim, Gi-Suk Doh, Jae Seong Yang, Do Hyeon Jeong, Sung Won Choi, and Kee Hyun Choi. Green Biotech Ltd., 45-70, Yadang-ri, Gyoha-myeon, Paju, Gyeonggi-do, Korea

A biocontrol agent, GBA-0927, was isolated from oriental orchid's natural habitat soils infected with *Fusarium oxysporum* f. sp. *cattleyae*. And they were screened and selected on basis of antifungal activity against *F. oxysporum* f. sp. *cattleyae* a pathogen to the *Cymbidium* spp. The candidate microorganism was identified as *Streptomyces kasugaensis* GBA-0927 on the basis of cultural and morphological characteristics. The fermentation broth of *Streptomyces kasugaensis* GBA-0927 had a broad antifungal spectrum against several plant pathogens, such as *Botrytis cinerea*, *Fusarium oxysporum*, *Magnaporthe grisea*, *in vitro*. An antifungal compound was isolated from *S. kasugaensis* GBA-0927 and identified as polyene macrolide antibiotics on the basis of various spectroscopic analyses including UV, IR, Mass and NMR. The molecular structure of the secondary metabolite produced by *S. kasugaensis* GBA-0927 was established as C₃₇H₅₉NO₁₄. (M.W. of 741.88).

B-32. The antibiotic effect of the secondary product purified from *Sclerotinia* sp. (BWC98-105) on the plant pathogenic bacteria. <u>Y. K. Hong</u>, J. N. Hyun, S. B. Song, B. C. Lee, M. G Chung, and S. C. Kim. National Yeongnam Agricultural Experiment Station, Milyang 627-803, Korea

Sclerotinia sp. (Isolate BWC98-105) causes stem blight, root rot in Leghum sp. and is presently being evaluated by author as a potential mycoherbicide for the control of white clover(Trifolium repens) originally. Two biologically active compound, Designated toxin I and toxin II were produced from culture filtrate of Sclerotinia isolate BWC98-105 in vitro. Bioassays with liquid cultured mycelia had shown that the Sclerotinia produces phytotoxins biologically active against some phytopathogenic bacteria. The toxin I and toxin II were purified by means of liquid-liquid extraction

and C18 open column chromatography(300 ×30 mm, i.d). To determin the purity, the purified toxin I and toxin II were analyzed by RP-HPLC. The flow rate was set at 0.7 ml/min. using linear gradient solvent system initiated with 15 % methanol to 85 % methanol in 50 min. with monitoring at 254 nm. Under these RP-HPLC conditions, the toxin I and toxin II were eluted at 3.49 and 4.13 min. respectively. Toxin I eluted at 3.49 min. had a unique antibiotic activity to X. oryzae pv. oryzae, B. glumae, R. solanacearum with others problematic bacteria on vegetable. In the leaf bioassay using toxin I (10 μ g a.i) against bacterial leaf blight of rice (X. oryzae) was tested. When the toxin I sprayed with 10 μ g a.i to 20-day-old rice seedlings, the lesion number of treated leaves were decreased to 2.1 lesions per leaf otherwise, untreated control showed 18.3 lesions per leaf. We concluded that the toxin I purified from culture extract of Sclerotinia sp. is a potential antibiotics for control agent of some phytopathogenic bacteria.

B-33. Comparison of chitin-binding domain (type 3), cellulose-binding domain (family II), and their double domain on binding capacity and chitinase activity. S. K. Park, S. H. Park, and I. J. Jung. Dept Environmental Agricaltural science, Sunchon National University, Sunchon, Chonnam, 540-742, Korea

Chitinase (Chi54) from *Chromobacterium sp.* strain C-61 was consisted of a catalytic domain and a type 3 chitin-binding domain (ChBD3). In this study, the ChBD3 was removed by deletion mutagenesis or replaced by a family II cellulose-binding domain (CBDII) of endoglucanase A from *Cellulomonas fimi*. The double domain was obtained by construction of CBDII in upstream of ChBD3. After expression in *Escherichia coli*, the proteins from the cytoplasmic fraction were purified using Rotofor cell. The binding affinity and chitinase activity of Chi54 decreased largely by removal of the ChBD3, but were restored by replacement of CBDII. Chi54 with a CBDII was similar to that with a ChBD3 in binding affinities to both chitin and cellulose, and chitinase activities to both soluble and insoluble chitin. These results suggest that ChBD3 and CBDII have similar function. On the other hand, Chi54 with a double domain exhibited higher affinity and activity on all substrates than that with single domain.

B-34. The amino acid residues (MxYD) at the C-terminal ends of 6-strands are important for catalysis of subfamily A catalytic domain. C. W. Kim, O. K. Kang, S. H. Park, and <u>S. K. Park</u>. Dept. Environmental Agricaltural science, Sunchon National University, Sunchon, Chonnam, 540-742, Korea.

The catalytic domain of family 18 chitinases was divided into subfamily A and B. The MxYD motif at the C-terminal ends of 6-strands in the subfamily A was replaced by QxYN in the subfamily B. To investigate function of MxYD in the subfamily A, the M, Y and D in chitinase (Chi54) of *Chromobacterium sp.* strain C-61 were replaced with the Q, D and N or W, respectively, by site-directed mutagenesis. Y348D (replacement of Tyr348 by Asp) and D349W (replacement of Asp349 by Trp) mutants showed no measurable chitinase activity like E273W lacking proton donor. On the other hand, M346Q (replacement of Met346 by Gln) and D349N (replacement of

Asp349 by Asn) mutants showed about 2% and 50% chitinase activity toward soluble chitin, respectively, compared with that of wild type. However, their chitinase activity toward insoluble chitin was about 5% of wild type. These results suggest that Tyr348 in Chi54 is essential for the catalytic reaction, and Met346 and Asp349 are important for chitinase activity but no essential for the catalytic reaction.

B-35. Enhancement of chitinase activity by site-directed mutagenesis in a *Chromobacterium sp.* strain C-61 chitinase. J. Y. Lee and <u>S. K. Park.</u> Dept. Environmental Agricaltural science, Sunchon National University, Sunchon, Chonnam, 540-742, Korea.

In experiments to find amino acids essential for catalysis, we found that replacement of Thr218 by Ser (T218S) increases activity of chitinase (Chi54) from *Chromobacterium sp.* strain C-61. Present study was conducted to find factors involved in the enhanced activity. Among bacterial chitinases belong to subfamily A, the amino acids corresponding to the Thr218 were most in Thr and followed by order of Ser and Ala. Thus, Thr218 was replaced with Ala (T218A), Asp (T218D) and Glu (T218E). The highest chitinase activity was detected in supernatant cultured for 5 days in wild type and T218S, 7 days in T218A and T218E, and 9 days in T218D. The chitinase activity in maximum culture period was about 1.5 fold higher in T218S than in wild type. However, the chitinase activity decreased into about 40% in T218A and T218E, and 13% in T218D compared with that of wild type. However, optimum pH and temperature on chitinase activity and stability were not different wild type from the mutated proteins.

B-36. Isolation and antifungal and anti-Oomycete activity of aerugine produced by *Pseudomonas fluorescens* strain MM-B16. J. Y. LEE¹, S. S. Moon², and B. K. Hwang¹. ¹Laboratory of Molecular Plant Pathology, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea, ²Department of Chemistry, Kongju National University, Kongju 314-701, Korea.

The MM-B16 which bacterial strain showed strong antifungal anti-oomycete activity against some plant pathogens was isolated from a mountain forest soil in Korea. Based on the morphological, physiological and biochemical characteristics, the bacterial strain MM-B16 was identical to Pseudomonas fluorescens> biovar III. The molecular formula of the antibiotic M16B isolated from the culture filtrates of Ps. fluorescens strain MM-B16 was deduced to be C10H11NO2S (M+, m/z 209.0513) by analysis of EI mass spectral data. Based on the NMR and IR spectral data, the antibiotic M16B was confirmed to have the structure of a thiazoline derivative aerugine, 4-hydroxymethyl-2-(2-hydroxyphenyl)-2-thiazoline. C. orbiculare, P. capsici and Pythium ultimum were most sensitive to the antibiotic M16B, with MICs of approximately 10 g/ml. However, no antimicrobial activity was found against yeasts and bacteria even at the concentration of over 100 g/ml. Treatment with the antibiotic M16B exhibited a significantly high protective activity against development of Phytophthora disease on pepper and anthracnose on cucumber.