D-47 Isolation of a chitin-degrading bacterium, *Cellulosimicrobium cellulans* CH-10 and its antifungal activity. Kwang Youll Lee, Han Woo Kim, Ok Ju Chun, Young Byung Yi, Soon Je Jung, Seon Woo Lee and Byung Ju Moon. Dong-A University, 840 Hadan2-dong, Saha-gu, Busan, Korea

A bacterial strain with colloidal chitin hydrolysis activity was isolated from tomato cultivated healthy soil at Taejeo, Pusan. The isolate was identified as *Cellulosimicrobium cellulans* CH-10 by analyzing its morphological, physiological properties and 16S rDNA sequences. *C. cellulans* CH-10 was shown to excrete chitinases into the culture supernatant when cultivated in a liquid culture containing colloidal chitin. After concentration of the culture supernatant by precipitation with ammonium sulfate, the induced chitinases was analyzed by in-gel chitinase assay using carboxymethyl-chitin-remazol brilliant violet 5R (CM-chitin-RBV) as a substrate. Three protein bands possessing chitinase activity were obtained with apparent molecular masses of about 33, 47 and 73 kDa, respectively. When *C. cellulans* CH-10 isolate was co-cultured with the fungal pathogen *Fulvia fulva* TF13, causing tomato leaf mold, in PDB media, the CH-10 isolate showed a potent antifungal activity against the pathogen. The microscopic analysis suggested that the chitinase produced from the CH-10 isolate might act on the cell wall of *F. fulva* resulting in malformation of fungal hyphae.

D-48 Cloning and expression of two chitinase genes from *Bacillus licheniformis* N-1 and *B. licheniformis* CH-1. Han Woo Kim, Kwang Youll Lee, Kwang Ryool Heo, Young Byung Yi Jae sung Nam, Seon Woo Lee and Byung Ju Moon. Dong-A University, 840 Hadan2-dong, Saha-gu, Busan, Korea

We cloned chitinase genes from bacterial isolates from upland soils, *Bacillus licheniformis* N1 strain showing high antifungal activity and CH-1 strain possessing chitinase activity. The CH-1 strain was identified as *Bacillus licheniformis* CH-1 by analysis of 16S rDNA sequences. The chitinase genes of the N1 strain and the CH-1 strain were cloned by PCR using PCR primers based on chitinase gene sequence of *B. licheniformis* TP1. Two chitinase genes exhibited 96% of identity of deduced amino acids sequence by Clustal W analysis. The nucleotide sequence of two genes revealed a single open reading frame encoding 598 amino acids with an expected molecular mass of about 66 kDa. The deduced amino acid sequence of chitinase gene appeared to have three functional domains, such as catalytic domain (amino acid residues 44 to 433), fibronectin typeⅢ like domain (amino acid residues 460 to 541) and chitin-binding

domain (amino acid residues 551 to 580). Recombinant *E. coli* Top10' harboring the cloned chitinase gene (pCHI-N1 and pCHI-1) showed chitinase activity on media containing colloidal chitin. Among two chitinases, N1 chitinase was overexpressed driven by T7*lac* promoter in *E. coli* BL21 by pET42-a vector. The reconstruction of biocontrol agent *B. amyloliquefaciens* A-2 to improve biocontrol efficacy is in progress by introducing the cloned chitinase gene into the strain A-2.

D-49 Antifungal activity of *Bacillus* sp. against various plant pathogens. Hui-Seon Jeong[1], Sang-Pyo Lee[1], Kwon-Jong Kim[1], Jong-Sang Cheong[2], Sun-Ju Moon[2], Kwang-Ho Cha[2], Hyun-gon kang[1], Min-Seob Yeo[1], Hyun-Sik Lim[1], Sang-Woo Kim[1] and Youn Su Lee[1] [1]College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea; [2]B.I.G Co., Ltd, Taejeon 306-230 Korea,

We conducted in vitro tests with *Bacillus* sp. to show the inhibition of growth of various plant pathogenic fungi. A wide range of antifungal activity of *Bacillus* sp. was found against the plant pathogenic fungi. Pathogenic fungi tested included *Alternaria solani, Colletotrichum gloeosporioides, Fusarium oxysporum, Phytophthora capsici, and Rizoctonia solani. Bacillus* sp. was the most effective to inhibit the growth of *Colletotrichum gloeosporiodes*, *Alternaria solani*, and *Rizoctonia solani*. As a result, *Bacillus* sp. was proved to be a potential biocontrol agent for tested plant pathogens. In addition, diluted *Bacillus* sp. broth was also suppressed the mycelial growth of nearly all test fungi. *Bacillus* sp. was the most effective fort the control of the mycelial growth of *Alternaria solani*.

D-50 Screening of a antagonistic actinomycetes against *Plasmodiophora brassicae* causing clubroot disease of Chinese cabbage. Chang-Guk Kim, Shun-Shan Shen and Chang-Seuk Park. Division of Plant Resources and Environment, Gyeongsang National University, Jinju 660-701, Korea.

Total of 681 actinomycetes isolates were collected from rhizosphere soils of various plant species growing in high mountain, forest fire area, or reclaim land. The isolates were proliferated in liquid media then mixed into pot mixer prior to inoculated in resting spores of *Plasmodiophora brassicae*. Chinese cabbage were planted in pathogen inoculated soil and evaluated the disease suppression of each isolate. In preliminary screening trials, 32 isolates showed significant disease reduction compare to