

IV. 세균 병학

D-01. Characterization of *Streptomyces scabies* from Common Scab Lesions on Carrot. Duck Hwan

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Streptomyces isolate from common scab lesions on carrots was characterized. Morphological and physiological characterization of the isolate established that it was closely related to *S. scabies*. This organism had spiral spore chains and grey spore mass color, produced melanin pigment on ISP6, 7 medium. It grew on agar media at pH5.0, used all nine carbon sources, and was pathogenic on carrot and potato. *S. scabies* is the first described bacterium which causes common scab disease on carrot in Korea.

D-02. Potato Blackleg Disease Caused by *Erwinia carotovora* subsp. *atroseptica*. Jun Sub Kim¹, Duck

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Bacterial disease of the potato plant caused by *Erwinia carotovora* subsp. *atroseptica* was observed in several areas in Korea. *E. carotovora* subsp. *atroseptica* induced black or dark brown basal stem rot to wilting leaves. Pathogens were identified based on the morphological, physiological and chemical characteristics. *E. carotovora* subsp. *atroseptica* is the first described bacterium which causes blackleg on potato in Korea.

D-03. Bacterial Soft Rot of Leek by *Erwinia carotovora* subsp. *carotovora*. Ki Sun Kim¹, Duck Hwan Park²

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Occurrence of soft rots was observed on leek grown in Kangnung, Korea. The symptoms began as a small water-soaked lesion, which enlarged rapidly in diameter. The tissue within the affected region became slimy, disintegrating into a mushy mass of disorganized cells. This eventually resulted in wilting and death of the aboveground parts of the leek. The causal organism was isolated from the infected lesions and was identified as *Erwinia carotovora* subsp. *carotovora* based on the morphological, physiological and biochemical characteristics, and on the results of the Biolog program. *E. carotovora* subsp. *carotovora* is the first described bacterium which causes bacterial soft rot on leek in Korea.

D-04. Soybean Leaves as a Site for Epiphytic Multiplication of Soybean Sprout Rotting Bacteria. Jae

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Bacterial population density on soybean leaves was $10^3 \sim 10^5$ CFU/cm². Bacterial population density were increased by progress of plant growth stage. Population density of soybean sprout rotting bacteria on

soybean leaves was $0\sim 10^3$ CFU/cm². Population density of soybean sprouts rotting bacteria were related to cultivating arear, but not related to plant growth stage. Cultivars and population density of soybean sprout rotting bacteria were less corelated, and varied by plant growth stages and plant parts. *Erwina cypripedii*, *E. carotovora* subsp. *carotovora*, *Xanthomonas campestris* pv. *glycines*, *Pseudomonas putida* biovar A, *Staphylococcus* sp., and *Micrococcus* sp. were identified as pathogenic bacteria causing soybean sprout rot. In generally population density of *E. cypripedii*, *E. carotovora* subsp. *carotovora*, *Micrococcus* sp., and *X. campestris* pv. *glycines* were high.

D-05. Population Density Changes of the Total Bacteria and Soybean Sprout Rotting Bacteria on Soybean Pods. Eun Jeong Lee, Kwang Seop Han¹ and Jae Eul Choi. Department of Agronomy, Chungnam Nat'l

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The total bacterial population density on soybean pods was $10^5\sim 10^6$ CFU/cm². The total bacterial population density were increased by progress of plant growth stage. Population density of soybean sprout rotting bacteria on soybean pods was $0\sim 10^3$ CFU/cm². Population density of soybean sprout rotting bacteria were related to cultivating arear, but not related to plant growth stage. Cultivars and population density of soybean sprout rotting bacteria were less corelated, and varied by plant growth stages and plant parts. *E. chrysanthemi*, *X. campestris* pv. *glycines*, *Staphylococcus* sp., and *Micrococcus* sp. was identified as pathogenic bacteria causing soybean sprout rots. In generally population density of *Micrococcus* sp., and *X. campestris* pv. *glycines* were high.

D-06. Bacterial Leaf Spot of *Ficus retusa* Caused by *Pseudomonas viridiflava*. Jae Kyung Cha, Eun Jeong Lee and Jae Eul Choi. College of Agriculture, Chungnam Nat'l University, Taejeon 305-764, Korea.

A new bacterial disease of *Ficus retusa* was found in Kwachon during March 1998. The symptoms were appeared as dark-brown spots on the margin of leaves that were enlarged to irregular in shape. The lesions are rapidly progressed by necrosis and sunken in leaves. Five bacterial isolates obtained from affected leaflets proved to be pathogenic to *Ficus retusa* by artificial inoculation, producing similar symptoms to those produced naturally. From this symptom, "bacterial leaf spot of *Ficus retusa*" was proposed as the name. The pathogen was aerobic, Gram negative and positive reaction in the test of production of green fluorescent pigment. The bacterium showed positive for grow on a minimal medium containing manitol, sorbitol, m-tartrate and D-tartrate but not sucrose, and produced a negative reaction for arginine dihydrolase, levan, oxidase and positive reaction for potato soft rot. On the basis of bacteriological characteristic and pathogenicity, the present isolates were identified as *Pseudomonas viridiflava*.

D-07. Two Pathogenic Bacteria, *Xanthomonas campestris* pv. *pruni* and *Erwinia nigrifluens*runi

Causing a Shot Hole of Peach and Plum. Jae Eul Choi and Eun Jeong Lee. Department of Agronomy, Chungnam Nat'l University, Taejeon 305-764, Korea.

In 1998, bacterial spots of peach and plum were found in Naju and Milyang. The disease cause small, circular to angular, water-solaked leaf spots, which dry to dark brown to purple spots and separate from the surrounding tissues, to form a "shot hole" . Where lesions are close together, the surrounding tissue yellow and leaf fall frequently occurs. On peach fruit, tan pinpoint spots appear, which crack to form pits on the fruit surface, often with associated gumming. On plum fruit, circular, large, greasy spots occur which darken to black lesions, with central cracking as the fruit enlarges. The pathogenic bacteria were isolated from the diseased leaf and branches of Stone fruit were identified as *Erwinia nigrifluens*, *Xanthomonas campestris* pv. *pruni* on the basis of bacterial characteristics. Ten bacterial isolates obtained from infected plants were divided into a genera from their diagnostic characters. Seven isolates of them were identified as *X. campestris* pv. *pruni* on the basis of their bacteriological properties. On the other hand, 3 isolates were also determined as *E. nigrifluens*. *E. nigrifluens* are the first description of bacteria which cause the diseases on peach and plum plants. These isolates possessed an ability to affect peach and plum tissues by needle-prick. The symptos caused by *E. nigrifluens* were hardly distinguished from those of *X. campestris* pv. *prunii*. In addition, it was observed that most of naturally infected plants were attacked by the 2 pathogenic bacteria at same time. From the reason mentioned above, we proposed to use a single common name "short hole of peach and plum." for the both bacterial diseases, hereafter.

D-08. Occurrence of Rice Bacterial Grain Rot by Weather Condition of Rice Heading Time.

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Odaebyue *et al.*, 21 rice varieties, were planted at 30 May and 15 June to examine the weather conditions of rice bacterial grain rot in 1998 and the percentages of diseased spikes were examined by the weather conditions of 7 days before and after heading time. Relationship between the rainy days in 7 days before and after heading time and the percentages of diseased spikes was expressed by $Y=0.0016X^2+0.39X-0.8168$ ($R^2=0.7979$, $N=42$), and disease occurrence was high in over 3 rainy days. Relationship between the percentages of diseased spikes and the average of the lowest temperature and the diurnal range of temperature in 7 days before and after heading time was expressed by $Y=0.0498X^2 - 1.9461X + 18.934$ ($R^2=0.8061$, $N=42$) and $Y=4.114 - 0.4977X$ ($R^2=0.6749$, $N=42$), respectively. Rice bacterial grain rot was mainly diseased when the rainy days were over 3 days, the average of the lowest temperature was over 23°C, and the diurnal range of temperature was under 7.5°C.

D-09. Detection of *Pseudomonas tolaasii* Causing Brown Blotch Disease on Mushroom by Nested PCR. Kyu-Sik Jeong¹, Heak-Inn Lee² and Jae-Soon Cha¹. ¹Chungbuk National University, San 48 Gaesin Dong, Cheongju, Korea 360-763. ²National Plant Quarantine Service, Seoul Office, 905 Mok5 Dong, Yangchun Gu, Seoul, Korea 158-055.

Pseudomonas tolaasii causes huge economic loss on mushroom production worldwide and brown blotch disease caused by the bacterium is a major limiting factor for oyster mushroom cultivation in Korea. Incidence of brown blotch disease on oyster mushroom is very hard to predict and affected much by environmental conditions, and once population of pathogen reaches high enough on mushroom cultivation bed the disease prevails and it is very difficult to control. Nested PCR method is developed to detect *P. tolaasii* at very low level. Two sets of PCR primers were designed from tolaasin gene which had cloned previously and nested PCR with the primers could detect less than 1 to 5 CFU of *P. tolaasii* from pure culture depending on age of bacterial culture and culture media. The nested PCR with sample containing 1,000 times more other bacterial cells than *P. tolaasii* still amplified the DNA specific to *P. tolaasii*. PCR method developed in this study can be use to monitor *P. tolaasii* at very low level from water and mushroom, and makes it possible to prevent from using contaminated water and treat mushroom before the pathogen population builds up to dangerous level.

D-10. Antibiotic Biosynthesis in *Pseudomonas fluorescens* MC07 is regulated by Quorum Sensing. Jinwoo Kim, Byoung Keun Park¹, Ingyu Hwang¹ and Chang Seuk Park. Division of Plant Resources and Environment, Gyeongsang National University # 900 Gazwa-Dong, Chinju 660-701, Korea; ¹Plant Protectants R.U., Korea Research Institute of Bioscience and Biotechnology Yusung P. O. Box 115, Taejon 305-600, Korea.

Many Gram-negative bacteria regulate expression of specialized gene sets in response to cell population density. This regulatory mechanism, called quorum-sensing, is based on the production by the bacteria of small, diffusible signal molecule called the autoinducer. The autoinducers are *N*-acylhomoserine lactones (HSL) which differ in the structure of their *N*-acyl side chain. We used a HSL-bioindicator, *Agrobacterium tumefaciens* NT1 (pDCI41E33) reporter strain to detect alkanoyl-HSLs with chain length ranging from C₄ to C₁₂. The NT1 reporter strain can detect *N*-3-oxooctanoyl-HSL prepared from culture extractions of *P. fluorescens* 2-79, *A. tumefaciens* NT1 (pTiC58 Δ *accR*), and *P. aureofaciens* 30-84. Previously, we described that the MC07 strain colonizes various plant roots and possesses antifungal activity. To determine the regulation of biosynthesis of antifungal compound of the bacterium, a mutant *MlacZ* 157 and 158 which lost the antifungal activity were isolated after screening 2,500 colonies generated by *Tn5lacZ* insertions. The *lacZ* gene expression inserted into 157 and 158 can be detected by incubation with culture extracts of *P. fluorescens* 2-79, *A. tumefaciens* NT1(pTiC58 Δ *accR*), and *P. aureofaciens* 30-84. In addition, fractions of *A. tumefaciens* NT1(pTiC58 Δ *accR*) extracted by ethylacetate increased the production of antifungal compound, indicating that the MC07 strain produces a *N*-HSL signal that regulates the production of antifungal compound. We found that the strain MC07 produces *N*-3-oxooctanoyl-HSL determined by thin layer chromatography analyses.

D-11. Seed Treatment with a Bacterial Antagonist for Reducing *Pythium* Root Rot of Barley. Jinwoo

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The objective of this study was to select promising bacterial strains from barley roots that suppress the root pathogens and enhanced seedling growth of barley and wheat. The plant samples were collected from 247 sites of 4 different locations throughout the southern part of the country. Total 5,000 colonies were picked up to examine the antagonistic to *Pythium* spp., *Rhizoctonia* sp., and *Fusarium* sp. Through the series of *in vitro* examination, 37 isolates were selected that significantly inhibit the fungal mycelial growth, including three *Pythium* spp. which isolated from the root rot lesion of barley. *In vitro* bioassay against *Pythium* spp. for 4 selected isolates (4-10, 88-7-2, 141-9, 159-9) were made and compared their disease suppression ability with *Pseudomonas fluorescens* 2-79, *Ps. aureofaciens* 30-84 from Washington State University and *Ps. fluorescens* B16 and MC07, *Paenibacillus polymyxa* E681 which are developed in our lab. The bacteria were applied to barley (*Hordeum vulgare* L. cv. 'Chinyang') seed and sowed in pot soil which naturally infested with *Pythium* spp. The experiments were conducted in growth chamber at 20°C and examined the disease occurrence and emergence rates. Some bacterial strains inoculated on the seeds significantly increased seedling stands and early growth of barley compared with untreated seeds in pathogen-infested soil.

D-12. Analysis of 16S rDNAs and 16/23S Spacer Regions of Phytoplasmas Occurring in Korea and

Japan. Hyung Moo Kim¹, M. Tanaka², Kui Jae Lee¹ and I. Matsuda². ¹Chonbuk Nat. Univ. Chonju, Korea. ²National Agriculture Research Center, Tsukuba, Japan.

To investigate relationships of phytoplasmal diseases occurring in Korea and Japan, including paulownia witches' broom (PaWB), mulberry dwarf (MD), sumac witches' broom (SuWB), rhus yellows (RhY), Jujube witches' broom (JuWB) and chestnut yellows (CY), 16S rDNA and 16/23S spacer regions of these associated phytoplasmas were analyzed. Total DNAs were extracted by modified CTAB method from young shoots of phytoplasma-infected plants collected from various places in Korea and Japan. DNA fragments comprising 16S rDNA and 16S/23S spacer regions (SR) were amplified by PCR, purified by gel filtration, and directly sequenced in both strands using 12 primers with an automatic fluorescence-based DNA sequencer. Sequence analysis revealed Japanese isolates of PaWB, MD and JuWB were identical with corresponding Korea isolates. But, SuWM in Korea and RhY in Japan were distinguished by one nucleotide position of sequence heterogeneity in the two rRNA operons. CY was not classified into any known 16S rRNA gene group and considered to represent a new group or subgroup of phytoplasma.