주제발표 초록

1. **Development of Sensitive PCR Assays for Detection of *Pseudomonas tolaasii* Causing Brown Blotch Disease on Oyster Mushroom.** Jae-Soon Cha[1], Hyouk-Inn Lee[2] and Kyu-Sik Jeong[1]. [1]Dept. of Agricultural Biology, Chungbuk National University, Gaeshin Dong, Cheongju, Chungbuk, Korea 361-763. [2]National Plant Quarantine Service, Seoul Office,

Bacterial brown blotch disease causes huge economic loss in oyster mushroom production in Korea. Control of the disease is very difficult because the environment of oyster mushroom cultivation is a favorable for the growth of pathogens and chemical control has a restricted application. The prevention of pathogen introduction into cultivation bed is believed to be the most important and practical method of managing the disease. A sensitive and pathogen-specific detection method makes it possible to prevent from using contaminated water, spawn, and other materials for oyster mushroom cultivation.

The four different PCR methods were developed for detection and identification of *Pseudomonas tolaasii* causing bacterial brown blotch disease on oyster mushroom in this study. In order to obtain PCR primers specific for *P. tolaasii*, the gene involved in production of tolaasin, which is known for the primary disease determinant of *P. tolaasii*, was cloned and its nucleotide sequence was determined. Primers were designed from the nucleotide sequence and the two sets of primers, Pt-1A/Pt-1D1 and Pt-PM/Pt-QM, were selected based on their ability of amplification and specificity for *P. tolaasii*.

PCR with Pt-1A/Pt-1D1 and Pt-PM/Pt-QM amplified 449bp and 249bp DNA, respectively, only from DNA of *P. tolaasii* when 17 species of *Pseudomonas* and other bacterial pathogens on crop plants were used for PCR assay. Nested-PCR with Pt-PM and Pt-QM followed by the first round PCR with Pt-1A and Pt-1D1 could detect 3 cfu of *P. tolaasii* per PCR reaction. Nested-PCR of bacterial cells captured with the primary anti-*P. tolaasii* antibody and magnetic bead cojugated with 2nd antibody can detect *P. tolaasii* successfully from the sample containing other bacterial cells 10,000 times more than *P. tolaasii.* When 57 water samples collected from oyster mushroom cultivation houses in different area were tested with the immunocapture-nested PCR, the *P. tolaasii*-specific DNA was detected from 20 water samples. This result indicates that 37% of water being used in oyster mushroom house was contaminated with *P. tolaasii.* The PCR assays developed in this study will provide valuable tools to manage the economically important disease.