

## **Diagnosis (84 ~ 85)**

**I-84 Atomic force microscopy where BT and NT meet: a platform technology for imaging and nanoscale morphometry of microbial cells.** K.W. Kim<sup>1</sup> and E.W. Park<sup>2</sup>. <sup>1</sup>National Instrumentation Center for Environmental Management, Seoul National University, Seoul 151-921, Korea; <sup>2</sup>Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea.

Imaging and nanoscale morphometry were performed with *Pseudomonas aeruginosa* and conidia of the rice false smut fungus *Ustilaginoidea virens* by atomic force microscopy (AFM). AFM imaging involves scanning a sharp tip located at the free end of a flexible cantilever over the specimen surface while sensing the interactive atomic force between the tip and the specimen surface. The topographic data obtained by AFM contain the entire x, y, z-coordinates of the specimen, which allows line or region profiles to be quantitatively analyzed. The surface of bacterial cells that had not been coated with metals could be observed under ambient conditions at the spatial resolution of conventional scanning electron microscopy. Fine structural details could be achieved to show spines on the conidial surface. Spines were not uniform in size and arrangement with a lateral diameter of 200 to 600 nm. Analysis of the surface profiles of conidia over 1.0×1.0 m<sup>2</sup> scan areas revealed a variety of root mean square roughness (78.2 nm), surface area (1.8 m<sup>2</sup>), and volume (239.6 nm<sup>3</sup>) from topographic images. These results suggest that AFM can provide an insight into a comprehensive understanding of microbial cells at the nanoscale level, opening a new venue in plant pathology and its related research areas.

**I-85 Molecular biological approach for single spore isolation of clubroot pathogen, *Plasmodiophora brassicae*.** S.J. Jang, C.S. Jang, H.G. Kim. Dept. of Agricultural Biology, Chungnam National University, Daejeon, 305-764, Korea.

PCR technique using *Plasmodiophora brassicae*-specific primers was used to distinguish whether a single spore had propagated or not within the tissue of host plants. Based on the verification using this PCR technique, the identification of pure culture in infected host plants was increased extremely. In this experiment, when single spores were inoculated at six kinds of the host plants including chinese cabbage, radish, kale, turnip and leaf mustard, only three of all 176 host plants formed visual small clubs. In the PCR assay using PbITS 1 and PbITS 2 primer set, however, *P. brassicae* was

detected from 13 out of 37 in root chinese cabbage, 17 out of 31 in radish, 8 out of 44 in chinese cabbage and 15 out of 30 in turnip, respectively. It means that the infection of single spore and the successful propagation within host tissue develop frequently than that it has been known. When used the PCR assay, *P. brassicae* which propagated by the single spore within tissue will be recovered more, and it will be possible to isolate new races that are weak pathogenic or grow slowly on each cultivar or cruciferous crops.