

Jutatape Watcharachaiyakup 2007: Study of Bacterial Wilt Disease of Corn in Thailand.
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Thirteen strains of bacteria which positive reaction with ELISA Agdia® kit specific for *Pantoea stewartii* were tested by standard detection method and compared with *P. stewartii* subsp. *stewartii* LMG2715; the type strain, *P. agglomerans* 4633, 4045, 20569 *Erwinia chrysanthemi* *E.carotovora* subsp. *carotovora* and *Escherichia coli* . The standard methods compose of gram staining, motility test, growth on nigrosine selective medium and salt tolerance. PCR detection by using specific primer to 16s-23s rRNA/ITS, *cpsD* and *hrpS* gene of *P. stewartii* subsp. *stewartii* were also tested. The bacteria were clustered by AFLP DNA fingerprint, carbon source utilization, soluble protein SDS-PAGE profile and 16s rDNA sequences were also compared. From the results indicated that these 13 bacterial strains were not *P. stewartii* subsp. *stewartii*. However, all strain except XE7 and W strains were identified as *P. agglomerans*. The result revealed that only PCR detection of *hrpS* gene was a specific detection method for *P. stewartii* subsp. *stewartii*.

PCR based detection techniques of *hrpS* gene as a target gene for detection of *Pantoea stewartii* subsp. *stewartii* from infected plant and seed sample namely direct-PCR, magnetic bead-PCR, ampli-disk PCR and real-time PCR were compared with ELISA for detection efficiency. The result indicated that direct-PCR is the best method based on fast, easy, sensitivity, reliability and low cost. The detection sensitivity of direct-PCR was in the range of 10^2 - 10^3 colony forming unit (cfu) per reaction in plant sample. For seed lot sample, sensitivity of detection was in the range of 0.02 – 0.007% of seed contamination and detection efficiency was increased from 33 to 83% by sub-sample seed detection. Furthermore, this detection efficiency was covered the rate of seed to seedling transmission and should be used as a standard detection method of this pathogen in Thailand.

Student's signature

Thesis Advisor's signature