

Rungnapha Thongkreng 2014: Development of The Rapid Detection Kit for Brown Spot Bacterium of Orchid (*Acidovorax avenae* subsp. *cattleyae*) Master of Science (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Miss Sujin Patarapuwadol, Ph.D. 77 pages.

Brown leaf spot symptoms were observed in commercial orchid orchards in Nakhon Pathom, Ratchaburi and Samut Sakhon provinces on *Vanda*, *Phalaenopsis*, *Cattleya* and *Mokara* orchids. Ten isolates of rod shaped, motile, Gram-negative bacteria were isolated from *Vanda* and *Phalaenopsis*. On King's medium B, colonies are round, convex with smooth margin in creme to beige color and no fluorescent pigment. Pathogenicity testing of these bacteria on *Vanda* and *Phalaenopsis* orchids by inoculating on wounded leaves each with a separate bacterial isolate, brown necrosis with water soaked lesions was developed on all the inoculated plants after 5 to 7 days whereas no symptoms developed on the negative control plants. The rapid bacterial identification using carbon utilization on 95 carbon sources, GN2 microplate with MicroStation™ ID system (Biolog Inc., Hayward, CA, USA), identified these bacterial as *Acidovorax avenae* subsp. *cattleyae*. Polymerase chain reaction was carried out with *Acidovorax avenae* specific primers, SEQ ID NO:1/SEQ ID NO:2 derived from the 16S-23S rDNA intergenic spacer region (ISR), were also generated an amplicon of 550 bp from pure cultures in all of 10 isolates. The results indicated that brown leaf spot in *Vanda* and *Phalaenopsis* orchids caused by *Acidovorax avenae* subsp. *cattleyae*.

A specific and sensitive, gold particle-based immunochromatographic test strip (Immuno-strip) for the rapid detection of *Acidovorax avenae* subsp. *cattleyae* was developed. Specific polyclonal antibody against *Acidovorax avenae* subsp. *cattleyae* was produced in rabbit using membrane protein complex (MPC) from virulent strain. The Immuno-strip tests with suspension of 10^8 cfu/ml of *Acidovorax avenae* subsp. *cattleyae* gave the positive reading within 5 minutes. The sensitivity of *Acidovorax avenae* subsp. *cattleyae* detection on orchid using Immuno-strip was at the minimum concentration of 10^4 cfu/ml.

Student's signature

Thesis Advisor's signature