THESIS TITLE : DEVELOPMENT OF SEROLOGICAL METHODS FOR DETECTION OF *Xanthomonas campestris* pv. *vesicatoria* (DOIDGE) DYE A CAUSAL AGENT OF BACTERIAL SPOT OF TOMATO

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ABSTRACT

The objectives of this study was to develop the serological detection method of *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye which is the causal agent of bacterial spot of tomato. Therefore thirty-three isolates of the bacterium were isolated from leaf spot disease samples collected from Khon Kaen, Roiet and Sakonnakorn provinces. Thereafter their pathogenicity on tomato plants cv. VF 134, morphology, physiology and biochemistry were assessed. The results revealed that all isolates were pathogenic to tomato plants, gram negative, rod shaped and aerobic. Moreover they were able to utilize glucose, galactose and mannose as carbon source and hydroly starch and pectate. Based on the characteristics as mentioned previously all isolates obtained were designated as *X. campestris* pv. *vesicatoria* group B strain.

X. campestris pv. *vesicatoria* (LKKU-7) was selected as representative for preparation of antigens including sonicated cell (sonicated Ag) and autoclaved cell (autoclaved Ag : 121C for 45 min.) The distinct protein bands of those antigens were demonstrated by sodium dodesyl sulfate polyacrylamide gel electrophoresis. When the antigens were used for antiserum production. The rabbit was injected intramuscularously by each antigen for five times with ten days interval. When indirect-ELISA was employed. Titer of antiserum immunized by sonicated Ag (soAg-As) was 1:2000 and titer of antiserum immunized by autoclaved Ag (auAg-As) was 1:1000. The minimum detection concentration was 10^5 cfu/ml and no cross reaction with seed saprophytic bacteria and *Xanthomonas campestris* parthovars except *X. campestris* pv. *campestris* was observed. When latex agglutination was employed optimal concentration of soAg-As and auAg-As was 1:200 and 1:100 respectively and minimum detection concentration was 10^7cfu/ml.

For detection of *X. campestris* pv. *vesicatoria*, two serological methods used were indirect-ELISA and latex agglutination test and only soAg-As was utilized. One hundred and ninty-seven diseased seedlings germinating from the inoculated seeds were determined for presence of the bacterium by indirect-ELISA and used of selective medium Tween B The result showed that positive reaction on indirect-ELISA occurred in one hundred and sixty-six seedling samples and the bacterial colony on selective medium Tween B could be recovered from one hundred and ninty seedling samples. But all fourty-five diseased leaves taken from Khon Kaen, Roiet, Karasin and Sakonnakorn provinces gave positive reaction to indirect-ELISA. To detection of the bacterium from tomato seed, twenty-five tomato seed lots from the farmer fields were employed. Twenty-three obvious that the bacterium from twenty-five seed samples were detected by indirect-ELISA and the bacterial recovery could be made with all samples by use of selective medium Tween B. For latex agglutination test, culture of thirty-eight isolates of *X. campestris* pv. *vesicatoria,* fourteen seed saprophyte bacteria, and twenty-one isolated of *Xanthomonas campestris* pathovars were investigated. Similarity with antiserum specificity test could be recorded thirty-two diseased leaves collected from Khon Kaen, Roiet, Karasin and Sakonnakorn were used. All samples were positive for latex agglutination test and used of selection medium Tween B.