THESIS TITLE : CLASSIFICATION AND DETECTION OF *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye A CAUSAL AGENT OF BACTERIAL SPOT OF TOMATO

AUTHOR : MISS BONGKOJPRAI SRIWILAI

THESIS ADVISORY COMMITTEE :

P. Sirithorn .....Chairman

(Assoc. Prof Dr. Pisan Sirithorn)

Sopone Wongkaew .....

(Dr. Sopone Wongkaew)

Weerasak Saksirirat .....

(Asst. Prof. Dr. Weerasak Saksirirat)

Abstract

A study was conducted to identify the causal agent of bacterial spot of tomato in the Northeast and to develop a proper detection method for the disease. Twenty seven diseased samples of tomato leaves and fruits were collected from three locations namely; (1) Tambon Pha Ku, Amphur Muang, Khon Kaen; (2) Vegetable Crop Experimental Station, Facultyof Agriculture, Khon Kaen university and (3) Amphur Phimai, Nakorn Rachasima. The samples were processed using a standard method for bacterial isolation and the

resulting organisms were further characterized by biochemical test, serology and reaction of specific hosts. The results showed that bacterial cells of all isolates were rodshaped and gram negative. Their colonies on yeast extract dextrose-$CaCO_3$ (YDCA) at35°c were yellow, circulated and convex. By the physiological and biochemical tests, all isolates were able to hydrolyse starch and liquefy gelatin slightly. The bacteria could produce hydrogensulfide and produce acid from carbohydrates; arabinose, cellobiose, galactose, glucose, mannose and trehalose. All isolates were pathogenic only to pepper and tomato among the eight species inoculated (pepper, tomato, tobacco, soybean, mungbean, cowpea, *Solanum nigrum* and *Datura stramonium*). Based on the morphological, physiological, biochemical and pathogenic properties the bacterium was identified as *Xanthomonas campestris* pv.*vesicatoria*.

For group and race classification the bacterium was inoculated into tomato cv. Sida, pepper cv. Early Calwonder (ECW) and three other isogenic lines; ECW 10-R, ECW 20-R, and ECR 30-R. It was found that all of the isolates could be classified as tomato group (Xcv T). By using Ouchterlony double diffusion test, all isolates could not be distinguished serologically.

Three detection methods: seedling symptom test, enzyme linked immunosorbent assay (ELISA) and use of a semi selective medium (Tween B medium) were employed to assess the recovery of *X. campestris pv. vesicatoria* from artificially inoculated commercial tomato seeds.

Bacterial suspension of various concentrations of 3.95x10, $3.95x10^{3}$, $3.95x10^{5}$, $3.95x10^{7}$, $3.95x10^{8}$ and $3.95x10^{9}$ cfu/ml were used in the assay. The results indicated that by seedling symptom test, a minimum bacterial concentration at $3.95x10^{5}$ cfu/ml could be seed-transmitted. When the bacteria were partially removed from the seed by extraction procedure, the treated seeds inoculated at $3.95x10^{7}$ cfu/ml were capable of producing minimum infected seedlings. The extract from all inoculated seeds in suspension was further used for detecting the bacterium by ELISA and Tween B medium. Results of the investigation showed that the extract from inoculated seed at $3.95x10^{5}$ cfu/ml was the minimum concentration for seed inoculation to give positive ELISA reading. The visible reaction required at least $3.95x10^{6}$ cfu/ml in the seed extract. When the Tween B medium was used, The bacterial colonies, a positive result could be obtained from extract of the seed inoculated at least $3.95x10^{3}$ cfu/ml. By considering the time consumed, specificity and efficiency the ELISA test is suggested to be a proper detection method at the preliminary screening.