

Damrongsak Arlai 2015 : Development of Hybridization Techniques for the Detection of *Salmonella* spp. in Pork. Doctor of Philosophy (Agricultural Research and Development), Major Field: Agricultural Research and Development, Faculty Agriculture at Kamphaeng Saen. Thesis Advisor: Assistant Professor Sujate Chaunchom, Ph.D. 129 pages.

The dissertation newly modifies the fluorescence in *situ* hybridization technique (FISH) to detect for *Salmonella* spp. in the pork industry. Herewith the study shows the method of the rapid detection of *Salmonella* in pork sample which was conducted by using optimization of cell wall permeabilizing conditions for Sal3 probe labeled at 3'-end with terminal transferase by FISH. The study results unveil that the optimum condition for cell permeation is using lysozyme at the concentration of 1 mg/ml dissolved in 10mM Tris-HCl, 5mM EDTA, pH 8.0, and incubation proceeds at 37 C° for 3 minutes. Furthermore the dissertation newly classifies the quality of *Salmonella* detection in pork by FISH into five levels, namely, 0 - 4 as poor to very good, respectively. The optimal condition gave the average score for FISH signal at level 4. Comparison to the results between the standard method ISO 6579 (2002) and the FISH method evaluated by the Kappa statistics equaled to 0.46. The results derived from both techniques were corresponding and accepted in the moderate range of standard values. The sensitivity (93.5%) and the specificity (66.6%) were achieved by FISH compared with culture. While, the results of FISH experiment were applied to *Salmonella* detection that contaminated levels higher than 3×10^6 cfu/ml onto pork, detection in this study is less time consuming (only 8 hour) and convenience for many samples testing compared to the bacterial culture method. Additionally, the dissertation firstly implements the method for detecting low numbers of *Salmonella* cells in pork samples by integrating "Catalyse Reporter Deposition or CARD" and the dot blot hybridization. The result of this method can detect 7.47 µg/ml of *Salmonella* DNA (expected cells concentration <3 cfu/ml) which is better than conventional methods of dot blot hybridization. And the study recommends these purposed methods are appropriate to apply in a conventional lab.

Student's signature

Thesis Advisor's signature