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THIDA SIRIKUL : RAPID DIAGNOSIS FOR DETECTION OF NON-TYPHOIDAL *SALMONELLA* IN FROZEN CHICKEN BY USING FLUORESCEIN LABELED PROBE (ECL SYSTEM). THESIS ADVISOR : ORASA SUTHIENKUL, Ph. D., ANONG PARIYANONDA, M. Sc., KLAI-UPSORN PONGRAPEEPORN, Ph. D., VARANYA SANGPETCHSONG, Ph. D.
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A rapid detection of *hns* gene encoding DNA binding protein of *Salmonella* using hybridization with the HNS-P oligonucleotide probe labeled with ECL (3' oligolabeling) was performed in 120 frozen chicken samples prepared for export from various frozen food plants in Bangkok during May 1995 to July 1997. Those frozen food samples had already been examined by the quality control laboratories of those plants. The sensitivity of the HNS-P probe was assessed by dot blot hybridization with genomic DNA extracted from *Salmonella typhimurium* RIMD 1985001 which detected a limit of detection of approximate 31.3 ng and 10^6 CFU/ ml. The specificity of the probe was determined by colony hybridization assay. Of 129 *Salmonella* strains were hybridized with the HNS-P probe, while 211 strains of other enteric bacteria were not hybridized. The results showed 100% specificity. For identification of *Salmonella* by conventional methods, no *Salmonella* was detected in all 120 direct frozen chicken samples (direct method), but it was found that *Salmonella* was positive in 42% (50/120) of enriched samples on xylose lysine desoxycholate agar (XLD) and 63% (76/120) on modified semi-solid Rappaport Vassiliadis agar (MSRV). The distributions of the O serovars among the isolated salmonellae from the positive samples were in 5 groups and 14 different serovars. Among them, the most common serovar found in the samples was *S. enteritidis* followed by *S. virchow*, *S. hadar*, *S. blockley*, *S. amsterdam*, *S. paratyphi* B biovar java, and *S. heidelberg* etc. These 290 colonies were further determined by colony hybridization. All gave positive reaction for *hns* gene but the DNA from both direct and enrichment samples did not give hybridization signal. In addition, the PCR test based on the *hns* sequence was developed for amplification of *hns* gene of *Salmonella* in frozen chicken samples after enrichment in selenite cystine broth (SCB). Amplified products were analyzed by gel electrophoresis and hybridization assay that gave positive results in 84% (96/120), and 88% (101/120) respectively. Therefore, the PCR followed by hybridization assay were successfully developed for rapid diagnosis of *Salmonella* with *hns* gene in frozen chicken samples.