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SUPORNWIT PUNGCHITTON: RAPID DETECTION OF *VIBRIO PARAHAEMOLYTICUS* IN FROZEN SHRIMP BY USING NESTED PCR. THESIS ADVISORS : ORASA SUTHIENKUL Ph.D., KLAI-UPSORN PONGRAPEEPORN Ph.D., KANOKRAT SIRIPANICHGON M.D., M.P.H., PRATANA SATITVIPAWEE M.P.H. 167 P. ISBN 974-664-233-2

Nested PCR-based assay with two sets of primers was developed for the detection of lecithin dependent hemolysin gene (*ldh*) of *Vibrio parahaemolyticus* directly in 103 frozen shrimp samples. All food samples were obtained from a frozen food factory in Chachoengsao province from April to May, 1999. The nested PCR assay produced a specific product size of 196 bp and detected the lowest genomic DNA of *ldh* reference strain, *V. parahaemolyticus* BG26 (*tdh*$^-$*trh*$^-$) at 1 fg corresponding to 1.7 cells per PCR reaction. All 21 strains of other *Vibrio* spp. and enteric bacteria did not give a specific DNA band of 196 bp but all tested *V. parahaemolyticus* strains did give positive band, indicating 100% specificity by nested PCR assay. It was demonstrated that 79.6% (82/103) of frozen shrimp enrichment samples were contaminated with *V. parahaemolyticus* using the conventional method and 88.3% (91/103) by the detection of *ldh* of *V. parahaemolyticus* in the enrichment samples using the primary PCR. The detection rates were significantly different between these 2 methods ($p=0.012$). Furthermore, the detection of *ldh* of *V. parahaemolyticus* directly from the samples by the nested PCR was 33.9% (35/103) and was significantly higher than that of the primary PCR (7.7%; 8/103)($p<0.001$). In contrast, using the conventional method, only one sample was found positive for *V. parahaemolyticus* with the count of 10^2 CFU/g of food sample, which was acceptable according to acceptable limit of *V. parahaemolyticus* by the criteria of International Commission on Microbiological Specification for Food (ICMSF). However, if other criteria of microbiological quality standards (such as total viable count, coliform counts and *E. coli* counts) were considered, 37.9%(39/103) of the samples were unacceptable. This study successfully developed the nested PCR assay for rapid detection (less than 8 hr) of *V. parahaemolyticus* in frozen shrimp samples without a prior enrichment step. However, enrichment of the frozen food samples before using PCR method was necessary to improve the detection of viable but nonculturable *V. parahaemolyticus*. Application of nested PCR assay could give a significant screening method for detection of *ldh* of *V. parahaemolyticus* contamination in frozen food as specificity and positive predictive value of the test, as compared with primary PCR, were as high as 85.7% and 91.4%, respectively.