Thesis Title	Rapid Detection of *Vibrio parahaemolyticus* Hemolysin Genes in Frozen Shrimp by Using Digoxigenin Labeled DNA Probes
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ABSTRACT

The study on rapid detection of thermostable direct hemolysin gene (*tdh*) and thermostable direct hemolysin-related hemolysin gene (*trh*) by using the digoxigenin-labeled PCR probes were carried out in 111 frozen shrimp samples prepared for export products from May 1995 to September 1996. The sensitivities of both probes were determined by dot blot hybridization with extracted genomic DNA of reference strains *V. parahaemolyticus* AQ4613 (tdh^+) and AQ4023 (trh^+). The minimal amount of extracted DNA detected by both probes was 125 pg (50ng/ml) and 10^6 colony forming unit (CFU)/ml each. The specificities of *tdh* and *trh* PCR probes were determined by colony hybridization assay. All known tdh^+ (49), trh^+ (10) and tdh^+trh^+(27) strains were hybridized with the digoxigenin-labeled *tdh* and *trh* PCR probes, while negative controls using 248 other strains of *Vibrio* spp. and enteric bacteria were not hybridized, thus giving 100% specificity. By the routine microbiological method, 64% (71/111) of

V. parahaemolyticus and 41.4% of other vibrios were isolated only from enrichment medium, alkaline peptone water (APW) with 3%NaCl (indirect method), but none of *V. parahaemolyticus* were detected directly in shrimp samples (direct method). In addition, a total of 364 isolates of *V. parahaemolyticus* and 119 isolates of other vibrios showed negative results for hemolytic activitives (TDH^-) with modified Elek test. Subsequently, all 483 isolates (including 364 *V. parahaemolyticus* isolates) also gave negative reactions for both *tdh* and/or *trh* genes. However, using the probes to detect the *tdh* and *trh* of *V. parahaemolyticus* directly from the samples and the enriched samples, we found positive results for *tdh* in 13.5% and 31.5%, respectively. Only one (0.9%) of the enriched samples was positive for *trh*. In comparison with the culture method, the *tdh* probe was able to detect the *tdh* gene of *V. parahaemolyticus* in 13.5% (direct) and 25% (indirect) of nonculturable samples for *V. parahaemolyticus*. Thus, the digoxigenin-labeled *tdh* and *trh* PCR probes were very sensitive to detect the hemolysin genes directly in frozen shrimp samples. It was emphasized here that the standard culture method recommended by FDA was not able to differentiate *V. parahaemolyticus* isolates with hemolysin genes (*tdh* and/or *trh*) and those without hemolysin genes. However, the digoxigenin-labeled *tdh* and *trh* PCR probes were successfully developed for rapid diagnosis of *V. parahaemolyticus* isolates with hemolysin genes (tdh^+ and/or trh^+) in frozen shrimp samples.