Thesis Title Detection of the Genes for Thermostable Direct Hemolysin and Thermostable Direct Hemolysin-Related Hemolysin of *Vibrio parahaemolyticus* by Digoxigenin Labeled Probes

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ABSTRACT

The study of digoxigenin-labeled DNA probes specific for the genes (*tdh* and *trh*) encoding the thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) of *V. parahaemolyticus* were carried out. The sensitivities of the probes were determined by dot blot hybridization with extracted DNA of the reference strains of *V. parahaemolyticus*, AQ 4613 (TDH^+) and AQ 4020 (TRH^+). The minimal amount of extractd DNA detected by the *tdh* and *trh* gene probes was 1 ng each. The specificities of the probes were determined by colony hybridization technique. The *tdh* and *trh* gene probes showed positive signals with all known 50 tdh^+ strains and all known 14 trh^+ strains, respectively. Both probes also gave positive results with all known 20 tdh^+-trh^+

strains. Among 180 tested strains of other *Vibrio* species and enteric bacteria, only 6 strains of *Vibrio hollisae* gave a weak hybridization signal. The probes were then used in a dot blot hybridization test for the rapid diagnosis of *V. parahaemolyticus* in 200 clinical fecal specimens. The crude DNA extracts from rectal swab in Cary-Blair transport medium as well as 4 hr culture in alkaline peptone water (APW) with 3% NaCl were dotted in parallel onto the membrane for dot blot hybridization assay. It was found that 50 fecal specimens were positive for *V. parahaemolyticus* by conventional method and their crude DNA extracts from both methods were also positive for their *tdh* gene only by dot blot hybridization assay. The hybridization signals of DNA samples from APW with 3% NaCl gave stronger signals than those from rectal swab. The other 150 fecal specimens were negative for *V. parahaemolyticus* as well as their genes. The strains of *V. parahaemolyticus* isolated from 50 positive specimens also showed positive for the *tdh* gene by colony hybridization assay. Therefore, the dot blot hybridization method with digoxigenin-labeled probes was successfully developed for rapid diagnosis of *tdh* and *trh* genes of *V. parahaemolyticus* directly from crude DNA extracts from clinical fecal specimens.