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PRASERT ROJLORSAKUL : DEVELOPMENT OF A SPECIFIC DNA PROBE AND PCR BASED TECHNIQUES FOR DETECTION OF *VIBRIO PARAHAEMOLYTICUS* THESIS ADVISOR : TIMOTHY W. FLEGEL Ph.D., VICHAI BOONSAENG Ph.D., WATANALAI PANBANGRED D.Eng., ORASA SUTIENKUL Ph.D. 142 p. ISBN 974-589-150-9

A DNA probe and a PCR method were developed for detection of penaeid shrimp pathogenic *V. parahaemolyticus*. From a genomic library, a probe named pVPA7 was selected and subcloned. It contained a 1.5 Kb species-specific DNA fragment which was selected by hybridization techniques for use as a DNA probe. This probe gave 100% specificity for all of the *V. parahaemolyticus* strains tested when tested with a wide range of *Vibrio* species and species representative of other bacterial genera including 126 strains of *V. parahaemolyticus* isolated from seafood. The sensitivity limit for *V. parahaemolyticus* was 10^5 CFU/ml by dot blot hybridization. After sequencing the 1.5 Kb fragment, primers VPAFOR3 and VPAREV1 showed good specificity for *V. parahaemolyticus* for a single 285 bp in PCR amplification tests. The specificity was 100% for the bacterial strains tested and sensitivity as 100 fg DNA and 4.0×10^3 CFU/ml or 20 cells of crude bacterial lysate in a PCR mixture. Southern blot hybridization of amplified products improved detection to as low as 0.1 fg DNA. PCR amplification was completely inhibited by 10^8 *V. parahaemolyticus* cells. There was no interference by contaminating bacteria (*E. coli*) at 10^7 cells. The lowest concentration of *V. parahaemolyticus* cells that could be detected in shrimp haemolymph was 4.0×10^3 CFU/ml or 20 cells per PCR reaction tube. However, *V. parahaemolyticus* cells at 10^8 did not inhibit the amplification in the haemolymph samples. Dot blot hybridization of amplified products improved detection to as low as 20 CFU/ml of *V. parahaemolyticus*. No hybridization occurred with PCR reaction mixtures from other bacteria in shrimp haemolymph.