Identification of *Vibrio parahaemolyticus* in shrimp (*Penaeus monodon*) using polymerase chain reaction

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*Vibrio parahaemolyticus* is a marine bacterium that occurs naturally in aquatic environments and seawater. In addition *V. parahaemolyticus* is a well known human pathogen causing gastroenteritis through consumption of raw or undercooked contaminated sea foods. Contamination of shrimp with *V. parahaemolyticus* is a serious issue and the detection needs a rapid and cost effective method. There is a great amount of microbiological techniques based on cultivation, isolation and serotyping of these microorganisms, which have the drawback of requiring several days work in order to evaluate the presence of a particular bacterium. On the contrary, molecular techniques and especially Polymerase Chain Reaction (PCR) can be applied for the purpose allowing result acquisition in shorter period. *V. parahaemolyticus* strains possess a regulatory gene, *toxR*, which is present in all the strains. Thus the PCR method targeted to the *toxR* gene can be used as a method for identification of *V. parahaemolyticus* at the species level. Strains carrying the *tdh* gene, encoding the thermostable direct hemolysin (TDH), or the *trh* gene, encoding the TDH-related hemolysin (TRH), or both genes are considered virulent strains. Shrimp samples were obtained from culture ponds of Puttalam district and were blended in alkaline peptone water with 3% NaCl and inoculated into three tenfold dilution (10^1, 10^2, and 10^3). Tubes were incubated at 36°C ± 1(37°C) for 24 hours. After incubation, tubes with turbidity were streaked on 3% Thiosulfate Citrate Bile salt Sucrose (TCBS) agar. Since several *Vibrio* species including *V. parahaemolyticus* grow on TCBS as green coloured colonies, it is necessary to identify them further using bio-chemicals in order to confirm the species. Suspected green colour colonies were used for the DNA extraction, PCR analysis and biochemical tests. Specific PCR for *toxR* gene detection gave positive results in which a band with 368 base pairs size appeared on the gel for some of the isolates that confirmed the presence of *V. parahaemolyticus*. All the isolates which gave positive results for *toxR* were examined for *tdh* and *trh* genes targeting chromosomal locus at 251 and 250 bp. Isolates which were tested for *tdh* and *trh* didn’t give positive bands. Therefore it can be stated that although the samples were positive for *toxR* they were not containing virulent genes and the strains which were positive are not virulent strains. Comparing with biochemical tests Polymerase Chain Reaction is found to be a faster, easier and more reliable method for the detection of *V. parahaemolyticus* in sea foods.