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

[1]M. Jeyakumar, [2]K.W.S. Ariyawansa, [2]P.H. Ginigaddarage and [1]M.S.W. De Silva
[1]Dept. of Food Science and Technology, Wayamba University of Sri Lanka, Sri Lanka [2]Post Harvest Technology Division, National Aquatic Resources Research and Development Agency, Crow Island, Sri Lanka

Vibrio parahaemoyiticus is a marine bacterium that occurs naturally in aquatic environments and seawater. In addition *V. parahaemoyiticus* 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. parahaemolitycus* strains possess a regulatory gene, *tox*R, which is present in all the strains. Thus the PCR method targeted to the *tox*R gene can be used as a method for identification of *V. parahaemolitycus* at the species level. Strains carrying the *tdh* gene, encoding the thermostable direct heamolysin (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 *tox*R 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.