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Note

Detection of *Vibrio parahaemolyticus* from saltwater fish samples by Vp-toxR PCR

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ABSTRACT

A total of sixty numbers of saltwater fish samples were collected and analysed by Vp-toxR PCR assay, directly from alkaline peptone water (APW) inoculated with samples and also by colony PCR from characteristic colonies on thiosulfate citrate bile salts sucrose (TCBS) agar. Vp-toxR PCR assay detected 5 (8.3%) samples positive for *Vibrio parahaemolyticus* directly from APW; whereas colony PCR detected 47 (78.3%) samples positive for the organism. Based on the results of the present study, it may be recommended to use colony PCR as a method suitable for detection of *V. parahaemolyticus* from saltwater fish samples.

Keywords: Alkaline peptone water, Colony PCR, Saltwater fish, TCBS agar, toxR PCR, *Vibrio parahaemolyticus*

Vibrio parahaemolyticus is a curved, rod-shaped Gram-negative halophilic bacterium that inhabits temperate as well as tropical estuarine, marine and coastal environment worldwide (Faruque and Nair, 2006). It was first identified as the cause of food borne illness in Osaka, Japan in 1950 following the illness of 272 individuals and 20 deaths (Fujino *et. al*., 1953). Raw or undercooked fish and seafood have been implicated as common vehicles of *V. parahaemolyticus* infection in human (Venkitanarayanan and Doyle, 2001) with manifestation of major clinical symptoms *viz*., wound infection and septicemia, gastroenteritis, bloody diarrhoea with characteristic "meat washed stool", abdominal cramps, nausea, vomiting, headache and low grade fever (Honda and Iida, 1993). *V. parahaemolyticus* was also reported to produce reactive arthritis (Tamura *et al*., 1993), fulminating necrotising fasciitis (Lim and Stebbings, 1999) and ear infections (Hornstrup and Gahrn-Hansen, 1993).

The *toxR* gene that is reported to be present in all strains of *V. parahaemolyticus*, is also a virulence associated gene. In the present study, a PCR targeting *toxR* gene of *V. parahaemolyticus* was standardised for rapid detection of organism from fishes. A total of 60 saline water fish samples comprising of bhetki (*Lates calcarifer*), parse *(Liza parsia)*, pabda (*Ompok pabda*), pomfret (*Pampus chinensis*) and lote (*Harpodon nehereus*) were collected aseptically from various established fish markets in Kolkata, India during December 2009 to February 2010. The samples were transported to the laboratory in ice packs and processed immediately. Gills and intestinal tracts were selected as sample sources, because of higher rate of isolation of *V. parahaemolyticus* from these sources, as evidenced by Das *et al.* (2009). Approximately 10 g of gill and intestine tissue samples cut into small pieces with a sterile scissors, were inoculated in enrichment broth (alkaline peptone water, APW) and incubated at 37 ^{0}C for 24 h. The enriched broth was streaked on TCBS (thiosulfate citrate bile salts sucrose) agar plates, incubated at 37 ^{0}C for 24-48 h and observed for the presence of characteristic non-sucrose fermenting colonies with green or blue centre.

PCR was performed in a total reaction volume of 25 µl, containing 2.5 µl of 10X PCR buffer, 200 µM of each dNTP, 25 pmol of forward (5'GTC TTC TGA CGC AAT CGT TG 3') and reverse (5'ATA CGA GTG GTT GCT GTC ATG3') primers (Kim *et al*., 1999), 1 unit Taq DNA polymerase, 5 µl of bacterial lysate and sterile de-ionised water upto 25 µl. Bacterial lysate, from enrichment broth and colonies on TCBS agar, was prepared as per Chai *et al.* (2007). Standardisation of PCR conditions was done using bacterial lysate of reference strain of

V. parahaemolyticus (Vp-Kx-V138), procured from NICED, Kolakata, India. The PCR amplification was standardised in a thermal cycler (Gene Amp PCR 9700; Applied Biosystems, USA) with initial denaturation at 95 ºC for 5 min, followed by 20 cycles at 94 ºC for 1 min, 63 ºC for 90 sec. and 72 ºC for 90 sec. Final extension was carried out at 72 ºC for 7 min. The amplified product (368 bp) was electrophoresed in 1.5% agarose gel (Biogene, USA) stained with ethidium bromide (0.5 µg ml^{-1}) and image was documented (Fig. 1) in a gel documentation system (Axygen, USA).

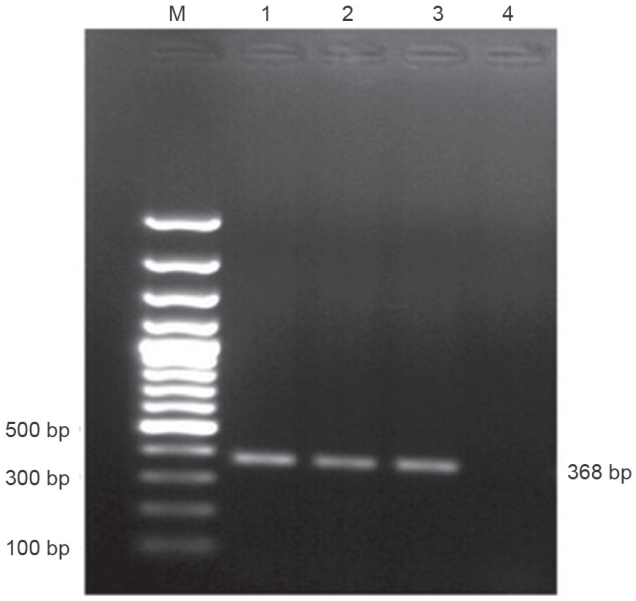

Fig. 1. Gel image showing PCR amplification of toxR gene of *Vibrio parahaemolyticus* from saltwater fish samples
M: 100 bp DNA ladder;
L1: Reference strain as positive control (368 bp)
L2 and L3: Fish isolates with positive amplicon
L4: Negative control

Out of the 60 samples screened for direct detection of *V. parahaemolyticus* from enrichment broth and colony PCR by Vp-toxR PCR, the PCR from enrichment broth detected only 5 (8.3%) samples to be positive for species specific *tox*R gene of *V. parahaemolyticus,* whereas colony PCR detected 47 (78.3%) samples to be positive. In contrast to the present findings, Deepanjaali *et al*. (2005) reported 93.8 % samples positive for *V. parahaemolyticus* after 18 h of enrichment while colony PCR could detect 91.8% of samples positive for *V. parahemolyticus* in oysters from estuaries of south-west coast of India. In another study, Dileep *et al*. (2003) analysed 86 samples of molluscan shellfish, water and sediment by *toxR* targeted PCR from enrichment broth and found 61.6% of the isolates positive by direct Vp-toxR PCR, whereas conventional isolation and biochemical method could identify only 28 (32.6%) samples as *V. parahaemolyticus* positive.

The present finding of the positive cases by colony PCR assay was strikingly higher than the direct PCR from enrichment broth, which could be attributed to the presence of some PCR inhibiting compounds in the APW broth or samples or to growth of other microbes in the broth. Thus it can be recommended that colony PCR method may be a more suitable method for detection of *V. parahaemolyticus* from saline water fish compared to direct detection from broth.

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