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Sequence variant *tdh* gene in environmental strains of *Vibrio parahaemolyticus*

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V. parahemolyticus is a natural inhabitant of coastal-marine environment. Food poisoning due to *V. parahaemolyticus* is associated with the consumption of raw or partially cooked seafood, especially the shellfish such as clams and oysters. Among the different virulence factors described in *V. parahaemolyticus*, chromosomal *tdh* and *trh* genes are well studied pathogenic determinants and their presence poses serious health risk to humans. The presence of these virulence factors has been found to correlate with the hemolytic toxins as evidenced in various mouse bioassays. Studies have shown that the detection rate of *trh* gene varies between 0% to 59.3% and *tdh* gene from 0% to 8.4% in different seafood and marine samples. Moreover, the detection rates of these genes were higher in clinical samples compared to the environmental and seafood samples. A Study was carried out to ascertain the pathogenic characteristics of *V. parahaemolyticus* from 140 samples comprising fish (40), shellfish (32), coastal sediment (23) and coastal water (45) from different fish markets and landing centers located in and around North Western Mumbai, Maharashtra, using *tdh* and *trh* targeted polymerase chain reactions (Honda et al., 1991; Okura et al., 2003). The *tdh*-specific PCR yielded non-specific amplifications visible as strong bands in the agarose gel and did not produce the expected amplicon size of 263 bp

(Fig. 1). To confirm the presence of *tdh* gene sequences in these amplicons, Southern blotting and hybridization was done using a biotin-labeled polynucleotide probe (Brown, 2001). The probe was prepared by PCR targeting *tdh* gene (263 bp)

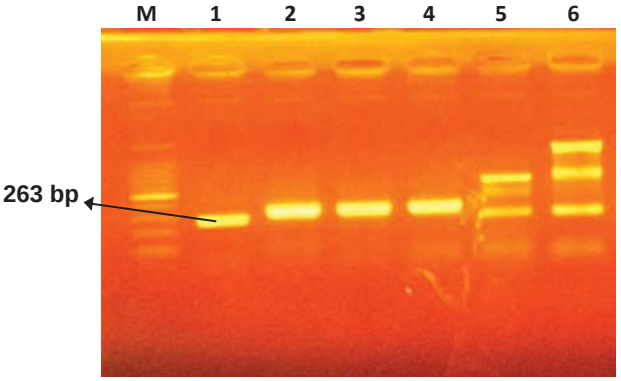

Fig. 1: Detection of tdh gene of V. parahaemolyticus
(Lane M: GeneRuler 100 bp (Fermentas, USA); Lane 1: Positive control; Lane 2-6: Amplification pattern obtained for *V. Parahaemolyticus* isolates)

from the reference strain of *V. parahaemolyticus* O3:K6. DNA bands were transferred onto nylon membrane by Southern blotting. Positive hybridization signals were obtained with three products confirming that these products were indeed from the *tdh* gene (Fig. 2).

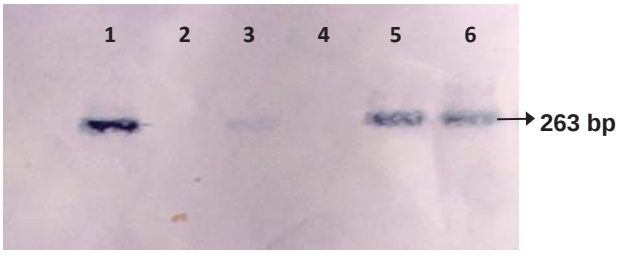

Fig. 2: Southern blotting and hybridization of **tdh***-gene products*
(Lane 1, 3, 5, 6: Positive for *tdh* gene; Lane 2, 4: Negative for *tdh* gene)

The *tdh* gene-specific primers are usually very specific and do not cross react with genes other than the *tdh* gene. Among the five isolates tested, three were found positive for *tdh* gene by southern blotting followed by hybridization using biotin-labeled polynucleotide probe. Lee and Pan (1993) have used this technique for the confirmation of *tdh* gene in *V. parahaemolyticus* strains. Sequence variant *tdh* gene of *V. parahaemolyticus* has been previously reported. Nishibuchi and Kaper, (1995) reported five *tdh* alleles in *V. parahaemolyticus,* namely, *tdh1* to *tdh5* with >96.7% sequence identity. Bhowmik et al. (2014) conducted phylogenetic analysis of 52 *tdh* and *trh* genes submitted to the GeneBank and suggested that there was a high level of sequence diversity in *tdh* and *trh* among *V. parahaemolyticus* strains.

The result suggested that seafood may harbor pathogenic *V. parahaemolyticus* possessing variant *tdh* genes which may go undetected by routine PCR using *tdh* gene-specific primers. Several recombination events such as insertion, deletion and duplication can result in alteration of sequences of a particular gene. Such events can inactivate a gene making the bacterium less pathogenic or result in the over expression of gene leading to enhanced pathogenicity. Altered nucleotide sequences alter the amino acid sequences and eventually the structure-function relationships of the protein in question, and these can have several implications on the activities of the protein and the physiology and virulence of the bacterium. It is necessary to further characterize the *tdh* gene in these isolates to understand the nature of sequence variation and their pathogenic significance.

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Carboxymethyl chitosan (CMCH): A water-soluble derivative of chitosan

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The structural uniqueness of chitosan facilitates the modification of its functional moieties which permits flexible manipulation of its biological and engineering properties. However, the poor solubility of chitosan at neutral pH poses practical difficulties in applications. To overcome this technological demerit, various chemical modifications have been suggested. Among these, carboxymethylation has often been applied to improve water solubility to chitosan. Fish Processing Division of ICAR-CIFT has standardised a cost-effective protocol for the production of Carboxymethyl chitosan at pilot scale. CMCH holds several bioactive and physicochemical properties, which are evensuperiorto those of native chitosan. Hence, itis considered as a promising candidate for biomedical applications such as drug carriers, antimicrobial material, gene delivery systems and tissue regeneration devices. As per our laboratory developed method, the properties of CMCH can be tailor-made to fit the requirements of these wider range of applications. In order to derive standardized combinations of process parameters to yield CM-chtosan with a defined set of properties for specific applications, a series of 18 trials were conducted. The properties of CM-chitosan incubated at various reaction temperatures ranging from 10-60ºC for different durations of 1-3 hrs were evaluated. The results indicated a distinct reduction in the solubility of CMCH below 40ºC, whereas only a marginal difference in solubility was observed between the samples incubated at