A Novel mRNA-targeted Method to Accurately Detect a Bacterial Pathogen: a Possible Tool to Improve Risk Assessment in the Area Where an Easy and Quick Quantitative Data Collection is Needed

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1. INTRODUCTION

The enteric infection is one of the most important infections among people in Southeast Asian countries. *Vibrio parahaemolyticus* is a marine bacterium and causes seafood-bone gastroenteritis in humans through consumption of seafood contaminated by virulent strains. However not all strains are virulent strains. The major virulence factors of *V. parahaemolyticus* are thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH). They are encoded by the *tdh* gene and *trh* gene, respectively, and virulent strains carrying these genes are rare (<1%) among the strains in the environment. In the investigation of *V. parahaemolyticus* infection, therefore, the isolated bacterial strains are usually examined for the presence or absence of these virulence genes by conventional polymerase chain reaction (PCR) methods. In the risk assessment of the seafood for *V. parahaemolyticus*, it is very important to assess the contamination of seafood by the virulent strains. An easy, rapid, accurate, and quantitative method for virulent strain detection is desirable in a local setting where generation of data is required with a limited facility within a limited time.

As pointed out by the previous paper in this session, such need was confirmed during the risk assessment study in southern Thailand. In this paper, we report a newly developed method that could be applied to improve the risk assessment study.

2. DEVELOPMENT AND PERFORMANCE OF TRC METHOD

We have developed a new method for detection of mRNA of the *tdh* and *trh* (including *trh*1 and *trh*2 subclass) genes of *V. parahaemolyticus* based on the principle of transcription-reverse transcription concerted (TRC) method. It is easy, rapid, precise, and quantitative and allows detection of specific mRNAs. This TRC method is a method of real-time monitoring of isothermal RNA sequence amplification with a fluorescent probe. The amplification reaction and its monitoring are carried out by using a simple automated system which is in a small closed vessel. The steps of the TRC method are a little complicated but the actual reaction is carried out in a small vial containing commercially available reaction mix. The unique features of the TRC method as compared with the PCR method are that the temperature of the reaction is constant (42°C) resulting in shortening of reaction time and that viable but not dead strains are detected.

For the quantification, the standard RNAs synthesized in vitro are used as calibrators. The sensitivity of the TRC method using the calibrators was at least 10^3 copies and detection time was 10 to 20 minutes for the calibrators in the range between 10^3 and 10^7 copies.

3. EVALUATION OF TRC METHODS FOR DETECTION OF VIRULENCE GENES OF *V. PARAHAEMOLYTICUS*

We examined by these TRC methods the total RNA preparations extracted from over 100 strains of *V. parahaemolyticus* carrying and not carrying these virulence gene for *tdh*-, *trh*1-, and *trh*2-specific mRNAs. The specific mRNAs were specifically and quantitatively detected. The detection times ranged from 10 to 19 min, 14 to 18 min, and 9 to 12 min, respectively. Relative levels of TDH (protein toxin) produced in *tdh* gene-bearing strains as determined by the immunological method correlated with those of the *tdh*-specific mRNA deteced by the TRC method.

4. DISCUSSION

The TRC methods for the detection of the virulence gene-specific mRNAs of *V. parahaemolyticus* are specific for each mRNA, sensitive (detecting 10^3 mRNA copies), quantitative (range, 10^3 to 10^7 mRNA copies), and rapid (10 to 20 min). The TRC system is contained in a small closed vessel. It automatically measures the reaction and the result is recorded in a laptop personal computer. For the preparation of total RNA from *V. parahaemolyticus,* we used a commercially available kit. It is possible to apply this TRC system for quantitative detection of *V. parahaemolyticus* carrying the virulence genes in seafood if total RNA extraction is secured. It will then be a very valuable tool for the risk assessment of *V. parahaemolyticus* in seafood in the countries where such a simple and reliable technology is urgently needed.

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