Novel Method for Screening Raw Food for Vibrio parahaemolyticus

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[INTRODUCTION]
Vibrio parahaemolyticus is a major food-borne pathogen that causes worldwide health problems (1) characterized by diarrhea, vomiting, and abdominal cramps through the consumption of contaminated raw or undercooked shellfish since V. parahaemolyticus was first isolated from shellfish implicated food poisoning in Japan (2). Prevention of V. parahaemolyticus contamination of seafood is an important public health concern.

One of the common methods for detection of V. parahaemolyticus in foods is the most probable number (MPN) procedure using selective enrichment with alkaline peptone water or salt peptone broth and selective chromogenic agar (TCBS) according to the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) method (3). This method (BAM-MPN) is cumbersome and requires at least 3 days to get a result (4, 5). This thermal red color development (TC) agar is difficult to distinguish visually from other strains of Vibrio mimicus and Vibrio cholera subspecies, and they can be confused with sucrose-fermenting bacteria forming yellow color colonies. The BAM-colony lift method (BAM-CL) is specific detection method for detection of the thermostable direct hemolysin (Dh) gene of vibrio strains. Pathogenic V. parahaemolyticus has not been as frequently detected in foods as it has been in clinical samples (7). The Compact Dry VPTM (CDVPTM) method uses the Compact Dry VTM system (6) with the culture medium (peptone, sodium chloride, bile salts, antibiotics, and chromogenic substrates) and a gelling agent, and has been developed to simplify and rapidly screen raw seafood for total V. parahaemolyticus. The CDVPTM plates are sterilized by the electron beam and ready to use for cultivation of total V. parahaemolyticus. The CDVPTM method becomes a reducing in the working hour and the labor cost because of not necessary for preparing media. The CDVPTM method was compared with BAM-MPN and BAM-CL methods because of injured cells, and BAM-CL was used for molluscan shellfish and fresh raw fish because it was thought that it did not contain the injured bacteria. The aim of this study was to evaluate the CDVPTM to adapt to screening raw seafood for total V. parahaemolyticus.

[METHODS AND MATERIALS]
Method comparison study.

The CDVPTM method was compared with BAM-MPN for frozen raw salmon (salmon and scallops) and with BAM-CL for fresh raw salmon (tuna and oysters). BAM-MPN was used for the processed product because of injured cells, and BAM-CL was used for molluscan shellfish and fresh raw fish because it was thought that it did not contain the injured bacteria. Each sample was purchased from retail stores in Japan. All seafood was confirmed negative for V. parahaemolyticus by the FDA-BAM method before artificial contamination. According to investigation of 11 V. parahaemolyticus foodborne outbreak cases in Japan (7), food isolation level were as follows: 3 cases were < 10 CFU/g, 6 cases were 100 - 1000 CFU/g, and 2 cases were > 1000 CFU/g. Therefore, artificially contaminated seafood samples were selected and prepared at three contamination level (low: log 1-2 CFU/g, intermediate: log 2-3 CFU/g, high: log 3-4 CFU/g). Each contamination level was approximately 1 log higher than the previous. For strains of V. parahaemolyticus, RSI0 221197 for tuna and RSI0 231075 for salmon from the Research Institute for Microbial Diseases (Osaka University, Japan), ATCC 7006 for strains from American Type Culture Collection (ATCC, USA), and DSM 15780 for scallops from Bio Medical Laboratories, Inc. (Saitama, Japan) were used separately for each inoculated food type. Five 50 ml frozen salmon, four raw scallops, and fresh raw tuna each 450 ml phosphate buffered saline solution (PBS, Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) was blended for 1 min at 8000 rpm. Twelve oyster meats were pooled into a sterile blender jar. An equal volume of PBS was added, and samples were blended for 90 sec at 14000 rpm.

[RESULTS AND DISCUSSION]

According to the results of the examination with the CDVPTM and FDA-BAM methods for V. parahaemolyticus for each artificially contamination level, there was no significant difference between the CDVPTM and FDA-BAM methods by one-way ANOVA (P > 0.05). The difference was not observed in the results that each strain was being studied for one strain for one type of seafood. The r² between the CDVPTM and BAM-CL methods were 0.99 for fresh raw tuna (Fig. 2) and 0.95 for fresh raw oysters (Fig. 3). The r² between the CDVPTM and BAM-MPN methods were 0.95 for frozen raw salmon (Fig. 4) and 0.95 for fresh raw scallops (Fig. 5). For all comparisons, the slope and intercept values, as determined by linear regression analysis, were close to 1.00 and 0.00, respectively. The CDVPTM yielded consistent results with each inoculum level by comparison testing. The detection limit of CDVPTM method is 10 CFU/g. The CDVPTM method is easier to use without technical expertise than BAM-CL. The shelf life of the CDVPTM is one and half year at room temperature after manufacturing. The CDVPTM is for screening raw seafood for V. parahaemolyticus on the field and provide food safety for raw seafood.

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Figure 1. Colony formation of V. parahaemolyticus on Compact Dry VPTM plates are sterilized by the electron beam and ready to use for cultivation of total V. parahaemolyticus.

Figure 2. Correlation between Compact Dry VP and FDA BAM Method: Colony Lift for V. parahaemolyticus in fresh raw tuna.

Figure 3. Correlation between Compact Dry VP and FDA BAM Method: Colony Lift for V. parahaemolyticus in frozen raw salmon.

Figure 4. Correlation between Compact Dry VP and FDA BAM Method: MPN for V. parahaemolyticus ACCC 27909 was incubated at 35C for 20 h.

Figure 5. Correlation between Compact Dry VP and FDA BAM Method: MPN for V. parahaemolyticus in frozen raw scallops.