養殖マアジにおける腸炎ビブリオの検出

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Short Paper

Occurrence of *Vibrio parahaemolyticus* in the Cultured Japanese Horse Mackerel *Trachurus japonicus*

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Abstract: This study was undertaken to examine the occurrence of *Vibrio parahaemolyticus* in the cultured Japanese horse mackerel *Trachurus japonicus*. *V. parahaemolyticus* was detected in 20 to 80% of fish specimens collected in the warmer season with mean densities of 3.6×10^2 to 5.4×10^3 MPN/100 g. In water samples, low densities of *V. parahaemolyticus* (36 MPN/100 *ml*) were detected at water temperatures of 22.8－27.5℃. PCR amplification was performed for four strains of *V. parahaemolyticus* isolated from the fish, and all isolates examined were an avirulent type (*toxR* gene-positive and *tdh*- and *trh*-negative).

Key words: *Vibrio parahaemolyticus*; Japanese horse mackerel; *Trachurus japonicus*; Vp-*toxR*

Vibrio parahaemolyticus is a marine bacterium that is known to be responsible for vibriosis in marine animals such as abalone, crustaceans and fish (Buller 2004). This bacterium is halophilic and widely distributed in seawater, plankton and fish in both tropical and temperate zones (Colwell 1984). Taken together, these attributes increase the likelihood of vibriosis in marine animals, along with human gastroenteritis. In addition, the Japanese horse mackerel *Trachurus japonicus* is a commercially important aquaculture species and 3,000 to 7,000 tons are cultured annually in Japan, almost all of which is consumed raw. This study was therefore conducted to clarify the occurrence of *V. parahaemolyticus* in the intestinal tracts and gills of cultured horse mackerel.

A total of 65 specimens of Japanese horse mackerel (five fish a month), weighing from 108.5 to 182.0 g, were supplied from a fish farm in Uchiura Bay, Shizuoka Prefecture during the period from October 2002 to September 2003, and in September 2006. Surface seawater samples were concomitantly collected near the fish farm. Both fish specimens and seawater samples were cooled in ice and processed within 5 h after collection. Fish specimens were dissected aseptically to remove the intestinal tracts of the fish. Intestinal contents and gills were separately homogenized in nine volumes of 2% NaCl-phosphate buffered saline (PBS; pH 7.4). The *V. parahaemolyticus* density was estimated using the most probable number (MPN) method according to a manual of Japan Food Hygiene Association (1998). Moreover, representative strains were then assayed using PCR detection for the Vp-*toxR*, *tdh* and *trh* genes (Bilung et al. 2005).

V. parahaemolyticus densities in seawater samples and fish specimens collected during the period from October 2002 to September 2003 and September 2006 are shown in Table 1. Water temperatures ranged from 15.2℃ in January to 27.5℃ in September 2003. Low densities (36 MPN/100 *ml*) of *V. parahaemolyticus* were detected in October 2002, and August and September 2003 (water temperatures of 22.8－27.5℃). This bacterium was detected in only three out of 65 gill samples at a mean density of 3.6×10^2 MPN/100 g during the investigation period. In addition, *V. parahaemolyticus* was detected in 17 out of 65 intestinal samples with mean densities of 3.6×10^2 to 5.4×10^3 MPN/100 g. Especially, in the warmer season (October 2002, and May through September), 20 to 80% of the intestinal samples possessed this bacterium. These observations for *V. parahaemolyticus* densities in the fish specimens and seawater sampled in the warmer season corroborated those in the review of Colwell (1984).

Moreover, four *V. parahaemolyticus* isolated in September 2006 were assayed for *toxR*, *tdh* and *trh* genes by PCR. The *toxR* gene is involved in the regulation of toxin production in numerous *Vibrio* species, and Kim et al. (1999) established a *toxR*-specific PCR protocol for the detection of *V. parahaemolyticus*. Four isolates examined were all *toxR* gene-positive and *tdh*- and *trh*-negative. The result of *toxR*-PCR assay suggest that *V. parahaemolyticus* isolates were correctly identified by the pheotyptic characterization in this study.

V. parahaemolyticus is considered to consist of two

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Table 1. *Vibrio parahaemolyticus* densities in gill and intestinal samples of Japanese horse mackerel, along with seawater samples

Sampling Date	Water temperature (℃)	Seawater (Log MPN/100 *ml*)	Gills (Log MPN/100 g)	Intestinal tracts (Log MPN/100 g)
22 Oct. 2002	22.8	1.56	ND*	2.56 (20)**
11 Nov. 2002	16.0	ND	ND	ND
16 Dec. 2002	19.5	ND	ND	ND
14 Jan. 2003	15.2	ND	ND	ND
17 Feb. 2003	15.8	ND	ND	ND
10 Mar. 2003	17.0	ND	ND	ND
19 Apr. 2003	23.1	ND	ND	ND
19 May 2003	24.2	ND	ND	3.36 (20)
10 June 2003	24.7	ND	ND	2.89 ± 0.30 (60)
14 July 2003	26.0	ND	ND	3.09 ± 0.58 (60)
20 Aug. 2003	27.1	1.56	ND	3.73 ± 0.45 (80)
17 Sep. 2003	27.5	1.56	ND	3.57 ± 0.41 (80)
19 Sep. 2006	27.0	NT***	2.56 (60)	2.56 (20)

* Not detected.
** Mean ± SE when present (occurrence, %).
*** Not tested.

types, virulent and avirulent, with the former type possessing thermostable direct hemolysin (TDH) and/or TDH-related hemolysin (TRH). *Tdh* and *trh* genes are involved in the production of TDH and TRH, respectively, both of which are considered to be an important virulence factor (Tada et al. 1992). The finding that these genes were not detected in four *V. parahaemolyticus* isolated from the Japanese horse mackerel in this study means that these were all the avirulent type. However, marine environments provide a habitat where vibrios can be exposed to high levels of gene transfer by transduction, and consequently, putative transfers of virulence factor genes like *tdh* and *trh* can occur between marine bacteria (Nishibuchi and Kaper 1995). The possibility therefore exists that avirulent types of *V. parahaemolyticus* occurring in cultured fish have the potential to become virulent and cause gastroenteritis in marine animals and humans. Surveillance for this bacterium in cultured fish species should thus always be conducted to prevent the outbreaks of this bacterium.

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References

Bilung, L., S. Radu, A. Bahaman, R. Rahim, S. Napis, M. Ling, G. Tanil and M. Nishibuchi (2005) Detection of *Vibrio parahaemolyticus* in cockle (*Anadara granosa*) by PCR. *FEMS Microbiol. Lett.*, **252**, 85-88.

Buller, N. B. (2004) Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual. CABI Publishing, Cambridge, MA, USA, 361 pp.

Colwell, R. R. (1984) "Vibrios in the Environment", John Wiley and Sons, New York, 634 pp.

Japan Food Hygiene Association (1998) Standard Methods of Analysis in Food Safety Regulation. Japan Food Hygiene Association, Tokyo, 736 pp. (in Japanese).

Kim, Y., J. Okuda, C. Matsumoto, N. Takahashi, S. Hashimoto and N. Nishibuchi (1999) Identification of *Vibrio parahaemolyticus* strains at the species level by PCR targeted to the *toxR* gene. *J. Clinic. Microbiol.*, **37**, 1173-1177.

Nishibuchi, M. and J. B. Kaper (1995) Thermostable direct hemolysin gene of *Vibrio parahaemolyticus*: a virulence gene acquired by a marine bacterium. *Infect. Immun.*, **63**, 2093-2099.

Tada, J., T. Ohashi, N. Nishimura, Y. Shirasaki, H. Ozaki, S. Fukushima, J. Takano, M. Nishibuchi and Y. Takeda (1992) Detection of the thermostable direct hemolysin gene (*tdh*) and the thermostable direct hemolysin-related hemolysin gene (*trh*) of *Vibrio parahaemolyticus* by polymerase chain reaction. *Mol. Cell. Probes*, **6**, 477-487.