A Fluorogenic Assay for the Rapid Detection of Some *Vibrio* Species Including *Vibrio parahaemolyticus* in Foods

(Received June 27, 1989)

Takahisa MIYAMOTO^{*1}, Yea-Ing SHEU^{*1}, Harufumi MIWA^{*2}
and Shoji HATANO^{*1}

(^{*1}Department of Food Science and Technology, Faculty of Agriculture, Kyushu University:
6-10-1, Hakozaki, Higashi-ku, Fukuoka, Japan; ^{*2}Ajinomoto Co., Inc.:
1-5-8, Kyobashi, Chuo-ku, Tokyo, Japan)

A rapid assay method for some *Vibrio* species including *Vibrio parahaemolyticus* was developed. The assay involved the enrichment culture of *Vibrio* species in BSP-HP medium (0.2% bacto-tryptone, 2% sodium chloride, 250 units/ml polymyxin B sulfate, 0.025% sodium hexametaphosphate, pH 8.5) and the specific measurement of intracellular trypsin-like activity by using the fluorogenic substrate benzoyl-L-arginine-7-aminomethylcoumarin. In BSP-HP medium, growth and trypsin-like activity of bacteria other than *V. parahaemolyticus*, *V. alginolyticus* and *V. harveyi* were suppressed. Although trypsin-like enzyme in food samples interfered with the fluorogenic assay, the interference could be overcome by the addition of 1 mM EDTA to the reaction mixture. Using this fluorogenic assay with commercial seafoods, 400 cells of *V. parahaemolyticus* per gram of food sample were detected within 8 hr. Trypsin-like activity measured by the assay was proportional to the number of *V. parahaemolyticus* determined by the conventional BTB teepol agar plating method (correlation coefficient $r=0.97$). In the medium, *V. alginolyticus*, one of the *V. parahaemolyticus*-allied bacteria, and *V. harveyi* also showed vigorous growth and high trypsin-like activity. A more specific culture condition for *V. parahaemolyticus* is required, and further investigation is in progress.

Key words: *Vibrio parahaemolyticus*; fluorogenic assay; trypsin-like activity; rapid detection; benzoyl-L-arginine-7-aminomethylcoumarin

Introduction

Food poisoning in Japan is most frequently due to *Vibrio parahaemolyticus*. Most of the *V. parahaemolyticus* food poisoning is caused by raw fish and shellfish¹⁾. The most common method to detect *V. parahaemolyticus* in marine specimens is a culture procedure which depends on selective media and sometimes requires enrichment for isolation from sparsely-infected materials²⁾. With this conventional method, *V. parahaemolyticus* detection requires from two to three days; therefore, there is a requirement for a rapid detection method for use in food quality control.

In recent years, a number of new methods have been developed to increase the efficiency and speed of food microorganism detection while reducing the labor³⁾. The fluorogenic

assay, using a fluorogenic substrate for the high-sensitivity detection of an enhanced enzymatic activity in bacteria, is one of these methods⁴⁾.

The present study was undertaken to develop rapid, specific, and more efficient detection assays for *V. parahaemolyticus* by using the fluorogenic substrate, benzoyl-L-arginine-7-aminomethylcoumarin, to detect intracellular trypsin-like activity.

Materials and Methods

Strains

Escherichia coli IFO 3301, *Salmonella typhimurium* IFO 12529, *S. enteritidis* IFO 3313, *Enterobacter aerogenes* IFO 13534, *Pseudomonas fragi* IFO 3458, *Staphylococcus aureus* IFO 3060 and *Bacillus brevis* IFO 3331 were obtained from the Institute for Fermentation, Osaka. *Campylobacter jejuni* JCM 2013, *Bacillus subtilis* JCM

1465, *B. circulans* JCM 2504, *B. cereus* JCM 2152, *B. pumilus* JCM 2508, *Lactobacillus lactis* JCM 1248 and *L. heterohiochii* JCM 1198 were obtained from the Japan Collection of Microorganisms. *Vibrio parahaemolyticus* WP-1, *V. parahaemolyticus* 46-11, *V. parahaemolyticus* 39-11, *V. parahaemolyticus* 39-3, *V. parahaemolyticus* 26-1, *V. parahaemolyticus* 27-2, *V. parahaemolyticus* 33-7, *V. parahaemolyticus* 33-8, *V. parahaemolyticus* 33-10, *V. alginolyticus* 10-1, *V. alginolyticus* 13-1, *V. alginolyticus* 14-1, *V. cholerae* FK, *V. damsela* FK, *V. vulnificus* FK and *V. harveyi* FK were obtained from Fukuoka City Institute of Public Health. *V. cholerae* non 0-1 NR, *V. fluvialis* NR and *V. furnissii* NR were provided by Dr. Seiichi Umesako, Nara Prefectural Institute of Public Health. *V. anguillarum* Ty 12, *Alteromonas communis* IAM 12914, *A. undina* IAM 12922 and *Aeromonas hydrophila* A 0111003 were kindly provided by Prof. Ushio Shimizu, Ocean Research Institute, University of Tokyo.

Fluorogenic substrate

The 4-methylumbelliferone (4-MU) derivatives shown in Table 1 were purchased from either Nacalai Tesque, Inc., or Polyscience, Inc. The 7-aminomethylcoumarin (7AMC) derivatives were obtained from Peptide Institute, Inc., Osaka. These fluorogenic substrates were dissolved at a concentration of 6 mM in dimethyl sulfoxide and kept at a temperature of -20°C .

Media

All of the media were sterilized in an autoclave. These media included PM broth (1% polypeptone, 1% meat extract, pH 7.0) and BSP-HP medium (0.2% bacto-tryptone, 2% sodium chloride, 250 units/ml polymyxin B sulfate, 0.025% sodium hexametaphosphate, pH 8.5).

Selection and measurement of a marker enzyme for *V. parahaemolyticus* detection

Various bacteria from slant cultures for preservation were inoculated in PM broth and cultured overnight at 30°C . This broth with 2% sodium chloride added was used to cultivate *V. parahaemolyticus*. To measure enzyme activity, the culture broth was diluted to $A_{660}=0.02$ with PM broth. When there was no bacterial growth,

undiluted culture broth was used to measure enzyme activity. Bacterial cells in 7 ml of the culture broth were collected by centrifugation ($1,400\times g$ for 10 min); 3 ml of 50 mM phosphate buffer (pH 7.5) including 0.01 mM fluorogenic substrate was added to the precipitated cell mass; 20 μl of toluene was added for lysis; and the reaction was allowed to proceed for 1 hr at 40°C . The reaction was stopped by addition of 1 ml of 1 M glycine buffer (pH 11.0) and after centrifugation ($1,400\times g$ for 10 min), the fluorescence intensity of the supernatant was measured with a Shimadzu RF-520 fluorospectrometer. The excitation wavelength was 360 nm and fluorescence intensity was measured at 450 nm. A control, including all components of the reaction mixture except the cultured bacterial cells, was assayed with each sample. The enzyme activity was expressed as the fluorescence intensity of the test sample relative to that of the broth control, which was set at 50.

Preparation of food samples

Five grams of each food sample was homog-

Table 1. List of Fluorogenic Substrates

Compound	Degradation enzyme
4-MU- α -L-arabinopyranoside	Arabinosidase
4-MU- β -D-galactoside	Galactosidase
4-MU- α -D-glucoside	α -Glucosidase
4-MU- β -D-glucoside	β -Glucosidase
4-MU- β -D-glucuronide	Glucuronidase
4-MU- α -D-mannopyranoside	Mannosidase
4-MU- β -D-xylopyranoside	Xylosidase
4-MU-phosphate, disodium salt	Phosphatase
4-MU-sulfate, potassium salt	Sulfatase
Bz-Arg-7AMC	Trypsin-like enzyme
Leu-7AMC	Arylaminopeptidase
Pyr-7AMC	Pyroglutamyl peptidase
Suc-Ala-Pro-Ala-7AMC	Elastase
Suc-Gly-Pro-7AMC	Post-proline cleaving enzyme
Suc-Leu-Leu-Val-Tyr-7AMC	Chymotrypsin-like enzyme
4-MU: 4-methylumbelliferone; 7AMC: 7-aminomethylcoumarin	

Table 2. Enzyme Activities of Various Bacteria

Strain	Activity														
	Ar	Ga	α G	β G	Gl	Ma	Xy	Am	Ch	El	Py	Po	Tr	Ph	Su
Control	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
gram-negative															
<i>V. parahaemolyticus</i> WP-1*	1.3	1.0	1.2	1.1	0.9	1.6	1.3	17.6	1.3	1.5	0.8	2.5	55.5	0.8	1.1
<i>C. jejuni</i> JCM 2013	1.3	63.2	12.0	5.2	1.0	4.1	1.2	5.5	3.3	1.4	1.5	1.2	1.7	1.0	1.0
<i>E. aerogenes</i> IFO 13534	3.8	66.0	1.7	4.1	1.0	1.2	3.6	6.3	1.8	1.5	1.7	1.1	2.0	6.3	1.0
<i>E. coli</i> IFO 3301	10.3	109.0	1.3	1.3	104.6	1.1	1.0	11.4	1.9	1.3	1.0	1.1	7.5	7.6	1.0
<i>P. fragi</i> IFO 3458	1.3	1.2	1.1	1.0	1.1	1.2	0.9	2.8	7.2	0.9	2.1	1.1	1.8	0.8	0.8
<i>S. enteritidis</i> IFO 3313	1.0	1.1	1.7	1.4	0.8	0.9	1.7	7.4	1.1	1.5	1.1	1.2	3.3	3.9	1.2
<i>S. typhimurium</i> IFO 12529	1.3	0.9	1.1	1.2	0.8	0.8	1.0	7.3	0.9	1.0	1.0	1.2	3.2	7.2	0.9
gram-positive															
<i>B. brevis</i> IFO 3331	1.0	1.2	1.1	1.0	1.2	1.0	1.7	2.1	2.5	1.4	3.1	1.6	1.9	1.1	1.1
<i>B. cereus</i> JCM 2152	1.1	1.3	26.0	1.1	1.1	1.2	1.3	3.3	2.1	1.0	1.3	1.4	1.3	7.5	1.0
<i>B. circulans</i> JCM 2504	38.3	30.8	60.6	18.8	1.1	16.8	175.0	2.3	1.4	1.2	1.5	1.2	1.3	1.0	1.0
<i>B. pumilus</i> JCM 2508	1.0	7.0	0.8	64.0	1.1	1.0	2.8	2.3	3.6	1.0	1.4	1.1	1.2	1.0	1.1
<i>B. subtilis</i> JCM 1465	1.3	3.7	123.9	28.9	1.0	1.4	28.0	2.2	209.8	1.4	2.7	1.2	1.3	1.0	1.0
<i>L. heterohiochii</i> JCM 1198	1.4	1.6	34.0	1.2	1.0	1.1	1.2	9.2	2.3	1.6	2.8	1.5	1.0	1.8	1.0
<i>L. lactis</i> JCM 1248	0.9	1.3	21.2	1.2	1.1	1.5	1.0	11.3	3.1	1.4	3.1	1.3	1.6	1.4	0.9
<i>S. aureus</i> IFO 3060	1.0	1.1	3.0	1.1	0.9	1.4	0.9	4.0	1.5	1.4	1.8	1.1	1.7	14.5	0.8

Medium: 1% polypeptone, 1% meat extract, pH 7.0.

* Medium: 1% polypeptone, 1% meat extract, 2% NaCl, pH 7.0.

Abbreviations: Ar, arabinosidase; Ga, galactosidase; α G, α -glucosidase; β G, β -glucosidase; Gl, glucuronidase; Ma, mannosidase; Xy, xylosidase; Am, arylaminopeptidase; Ch, chymotrypsin-like enzyme; El, elastase; Py, pyroglutamyl peptidase; Po, post-proline cleaving enzyme; Tr, trypsin-like enzyme; Ph, phosphatase; Su, sulfatase

enized with 45 ml of peptone water (1% polypeptone, 2% sodium chloride, pH 7.0) in a sterilized Waring blender (14,700 rpm for 2 min) and used as an undiluted sample solution²⁾.

Vibrio colony count

The sample solution was serially diluted with peptone water, and 0.1 ml of the diluted solution was spread evenly over BTB teepol agar (Nissui Pharmaceutical Co., Ltd.). After incubation at 35°C for 18 hr, typical colonies of *V. parahaemolyticus* and *V. alginolyticus* were counted²⁾.

Results

Determination of a marker enzyme to detect V. parahaemolyticus

Fifteen strains of food-poisoning bacteria and food-spoilage bacteria, including *V. parahaemolyticus*, were selected and cultured in PM broth. Each strain was incubated with the substrate shown in Table 1, and 15 different enzyme activities were measured using the fluorogenic assay. The results are shown in Table 2.

Arylaminopeptidase and trypsin-like activities were highest in *V. parahaemolyticus*. Arylaminopeptidase activity was present in all the bacteria tested, but trypsin-like activity was not observed in gram-positive bacteria. The results suggest that when *V. parahaemolyticus* is cultured in a selective-enrichment medium, arylaminopeptidase or trypsin-like activity can be used as a highly sensitive indicator, by using the fluorogenic assay.

Selective-enrichment medium to detect arylaminopeptidase and trypsin-like activities of V. parahaemolyticus

To obtain higher activities of intracellular arylaminopeptidase and trypsin-like enzyme in *V. parahaemolyticus*, enrichment media of 32 different compositions were tested, and BS medium (0.2% bacto-tryptone, 2% sodium chloride, pH 8.5) was found to increase the activity of the enzymes. Polymyxin B sulfate (250 units/ml) and 0.025% sodium hexametaphosphate (HP)⁵⁾ were added to the BS medium to suppress

Table 3. Arylaminopeptidase and Trypsin-Like Activities of Various Bacteria Grown in BSP-HP Medium

Strain (Kanagawa hemolysin, Serotype)	Growth (A_{660})	Activity	
		Arylamino.	Trypsin-like
Control	0	1.0	1.0
<i>V. parahaemolyticus</i> WP-1 (+)	0.08	101.0	175.0
<i>V. parahaemolyticus</i> 27-2 (+, K7)	0.07	109.0	166.0
<i>V. parahaemolyticus</i> 33-7 (+, K64)	0.07	46.0	190.0
<i>V. parahaemolyticus</i> 33-8 (+, K8)	0.09	38.0	63.0
<i>V. parahaemolyticus</i> 33-10 (+, K38)	0.10	75.0	121.0
<i>V. parahaemolyticus</i> 26-1 (−, K41)	0.10	81.0	148.0
<i>V. parahaemolyticus</i> 39-3 (−, K57)	0.10	62.0	82.0
<i>V. parahaemolyticus</i> 39-11 (−, K13)	0.08	97.0	153.0
<i>V. parahaemolyticus</i> 46-11 (−, K8)	0.10	58.0	110.0
<i>V. alginolyticus</i> 10-1	0.11	39.0	112.0
<i>V. alginolyticus</i> 13-1	0.11	29.0	107.0
<i>V. alginolyticus</i> 14-1	0.12	53.0	103.0
<i>V. anguillarum</i> Ty 12	0	54.5	1.0
<i>V. cholerae</i> FK	0	29.3	1.6
<i>V. cholerae</i> non 0-1 NR	0.01	16.8	0.9
<i>V. damsela</i> FK	0	13.1	1.0
<i>V. fluvialis</i> NR	0.02	130.0	1.2
<i>V. furnissii</i> NR	0	36.3	2.0
<i>V. harveyi</i> FK	0.02	60.0	156.0
<i>V. vulnificus</i> FK	0.04	36.6	1.0
<i>A. hydrophila</i> A 0111003	0	1.5	2.3
<i>A. communis</i> IAM 12914	0	2.0	1.3
<i>A. undina</i> IAM 12922	0	1.3	4.1
<i>C. jejuni</i> JCM 2013	0	1.3	1.1
<i>E. aerogenes</i> IFO 13534	0.04	41.6	8.5
<i>E. coli</i> IFO 3301	0	18.8	4.4
<i>P. fragi</i> IFO 3458	0	1.9	1.5
<i>S. typhimurium</i> IFO 12529	0	9.8	2.7
<i>S. enteritidis</i> IFO 3313	0	10.7	2.5
<i>B. subtilis</i> JCM 1465	0	1.1	1.0
<i>B. circulans</i> JCM 2504	0	1.2	1.6
<i>B. cereus</i> JCM 2152	0	1.0	1.1
<i>B. brevis</i> IFO 3331	0	1.2	1.2
<i>B. pumilus</i> JCM 2508	0	1.7	1.1
<i>L. lactis</i> JCM 1248	0	1.0	1.9
<i>S. aureus</i> IFO 3060	0	1.0	1.0

Various bacteria were inoculated in BSP-HP medium and cultured at 30°C for 16 hr. Arylaminopeptidase (Arylamino.) and trypsin-like activities were measured by the fluorogenic assay.

the growth of gram-negative bacteria other than *V. parahaemolyticus* and gram-positive bacteria, respectively (BSP-HP medium). In BSP-HP medium, the growth and the enzyme activities of various bacteria were measured, and the results are shown in Table 3. *V. parahaemolyticus*, regardless of its Kanagawa response or antigen type, exhibited high arylaminopeptidase and trypsin-like activities. *V. alginolyticus* and *V.*

harveyi also grew in BSP-HP medium and showed high enzyme activities. *V. cholerae*, *V. cholerae* non 0-1, *V. fluvialis*, *V. damsela*, *V. vulnificus*, *V. furnissii*, *V. anguillarum*, *Alteromonas undina*, *Escherichia coli* and *Enterobacter aerogenes* showed low trypsin-like activity though they exhibited high arylaminopeptidase activity. These results indicate that after being cultured in BSP-HP medium, species of *Vibrio* such

as *V. parahaemolyticus*, *V. alginolyticus* and *V. harveyi* can be specifically detected by measuring intracellular trypsin-like activity.

Fig. 1. Time course of trypsin-like activity production

V. parahaemolyticus cells were cultured at 30°C in a test tube containing 7 ml of BSP-HP medium. The culture was centrifuged at $1,400\times g$ for 10 min after a suitable interval. Trypsin-like activity of the precipitate was measured by the fluorogenic assay. Cell number inoculated into the medium is indicated to the right of each curve.

Incubation period required for *V. parahaemolyticus* detection

To determine the minimum incubation period for the detection of trypsin-like activity, 5 to 5×10^3 cells of *V. parahaemolyticus* were cultured in 7 ml of BSP-HP medium, and the enzyme activity was measured periodically. The changes in trypsin-like activity are shown in Fig. 1. With the inoculation of 5×10^2 or 5×10^3 cells of *V. parahaemolyticus*, the enzyme activity was detected after incubation for 4 hr, and with initial cell numbers of 5 and 50, the activity was detectable after incubation for 8 and 6 hr, respectively.

Trypsin-like activity of *V. parahaemolyticus*, *V. alginolyticus* and *V. harveyi* cultured in BSP-HP medium

Trypsin-like activities of *V. parahaemolyticus*, *V. alginolyticus* and *V. harveyi* were compared when various initial cell numbers (0 to 10^7) were inoculated in BSP-HP medium. Figure 2 shows trypsin-like activity of *V. parahaemolyticus*, *V. alginolyticus* and *V. harveyi* cultured in BSP-HP medium. In all the strains of *V. parahaemolyticus* and *V. harveyi*, the activity was proportional to

Fig. 2. Trypsin-like activity of *V. parahaemolyticus*, *V. alginolyticus* and *V. harveyi* grown in BSP-HP medium

V. parahaemolyticus, *V. alginolyticus* and *V. harveyi* were inoculated in BSP-HP medium (7 ml) at various cell numbers and cultured at 30°C for 6 hr. The culture broth was centrifuged at $1,400\times g$ for 10 min. Trypsin-like activity of the precipitate was measured by the fluorogenic assay.

Symbols: ●, *V. parahaemolyticus* WP-1; ▲, *V. parahaemolyticus* 33-10;
■, *V. parahaemolyticus* 26-1; ○, *V. alginolyticus* 10-1;
△, *V. alginolyticus* 13-1; □, *V. alginolyticus* 14-1;
◇, *V. harveyi* FK

the initial cell number. In *V. alginolyticus*, however, the activity of the three strains was variable, and in two of the strains it was as high as that of *V. parahaemolyticus*. No activity was detected in *V. alginolyticus* 10-1 even at the initial cell number of 10^5 . All the strains used gave the highest activity when the initial cell number was 10^7 . The results suggest that the cell number of various *V. parahaemolyticus* can be estimated by measuring trypsin-like activity after cultivation in BSP-HP medium, though the

selective-enrichment medium for *V. parahaemolyticus* needs to be improved.

Elimination of the interference by food-derived enzymes

It is assumed that enzymes in the food may interfere with the measurement of *Vibrio*-derived enzyme. In fact, when enzyme activities in oysters were measured, high arylamino-peptidase and trypsin-like activities were observed in the absence of *Vibrio* species. To avoid

Table 4. Effects of EDTA on Arylaminopeptidase and Trypsin-Like Activity of Oyster and *V. parahaemolyticus* WP-1

Sample	Activity			
	Arylaminopeptidase		Trypsin-like	
	None	1 mM EDTA	None	1 mM EDTA
Control	1.0	1.0	1.0	1.0
Oyster	121.0	110.0	3.9	1.3
<i>V. parahaemolyticus</i>				
6.4×10^4 cells/ml	1.4	1.1	1.1	1.1
6.4×10^5 cells/ml	2.2	1.2	2.5	2.9
6.4×10^6 cells/ml	11.6	2.7	18.4	16.9
6.4×10^7 cells/ml	122.0	27.8	90.0	120.0

Table 5. Detection of *V. parahaemolyticus* in Seafoods by Fluorogenic Assay and BTB Teepol Agar Assay

Sample	Fluorogenic assay (trypsin-like activity)	BTB teepol agar assay (cells/g)	
		<i>V. parahaemolyticus</i>	<i>V. alginolyticus</i>
Control	1.0	0	0
Adductor of shellfish	7.4	600	3,400
	48.2	7,700	5,400
Ark shell	1.0	100	<100
	1.4	100	<100
	50.4	12,000	<100
Boiled octopus	1.2	<100	<100
	3.2	600	<100
	101.5	27,000	<100
Dried young sardine	1.9	<100	280
	2.2	600	7,200
	25.9	8,900	3,800
Horse mackerel	1.7	400	300
	121.0	25,000	300
Sea bream	0.8	100	<100
	1.5	400	<100
	41.8	14,000	<100
<i>Spratelloides gracilis</i>	2.3	100	<100
	4.5	700	<100
	92.0	29,000	<100
Squid	19.2	3,600	12,000
	53.6	12,000	9,100

Fig. 3. Relationship between the cell number of *V. parahaemolyticus* in food and trypsin-like activity

V. parahaemolyticus was determined by the conventional BTB teepol agar plating method.

this problem, a specific inhibitor that inhibits the food enzymes but not *Vibrio* enzymes was required. Among the inhibitors tested, EDTA strongly inhibited the oyster trypsin-like enzyme but not the *Vibrio* trypsin-like enzyme (Table 4). EDTA did not inhibit the oyster arylaminopeptidase (Table 4). Thus, when 1 mM EDTA was added to the reaction mixture, there was no interference in the activity of *Vibrio* trypsin-like enzyme by the food trypsin-like enzyme, and therefore, the trypsin-like activity could be used as a specific indicator to detect some *Vibrio* species including *V. parahaemolyticus*.

Comparison between the fluorogenic assay and conventional BTB teepol agar plating

The fluorogenic assay developed in this study was used to detect *V. parahaemolyticus* from commercial fish and shellfish. The results were then compared with those obtained by the conventional BTB teepol agar plating method. Samples of commercial horse mackerel, boiled octopus, squid, blue sprat, sea bream, ark shell, dried young sardine and adductor of shellfish were inoculated with 0 to 10⁴ cells of *V. parahaemolyticus* per gram. Sample solution (1 ml) was added to BSP-HP medium (6 ml) and cultured at 30°C for 6 hr. Trypsin-like activity was measured by the fluorogenic assay. The results obtained by both methods are summarized in Table 5 and Fig. 3. A very strong correlation

was recognized (correlation coefficient $r=0.97$) between the colony count of *V. parahaemolyticus* and the trypsin-like activity measured by the present method. When the activity was over 2.0 (double that of the control), the sample was estimated to contain over 400 cells of *V. parahaemolyticus* per gram.

Discussion

Feng *et al.*⁶⁾ and Moberg⁷⁾ used glucuronidase as a marker enzyme for the detection of *E. coli*. The commercially available Biorapid E kit (Ajinomoto Co.) for rapid detection of *E. coli* measures the galactosidase activity of the bacteria after a selective enrichment culture. From the results with various microorganisms causing food poisoning and spoilage, very high glucuronidase and galactosidase activities were detected in *E. coli* (Table 2). The Biorapid B kit (Ajinomoto Co.) for total viable count contains several 7AMC derivatives of amino acids for detecting arylaminopeptidase activities of bacteria. In the present experiment, all bacteria tested exhibited arylaminopeptidase activity (Table 2). Our results support the utility of these enzyme tests for the detection of the bacteria described above. Phosphatase activity was detected in many gram-negative bacteria, *B. cereus*, and *S. aureus* (Table 2). By measuring the phosphatase activity, *S. aureus* is probably detected specifically when it is cultured in the enrichment medium that contains inhibitors of growth of gram-negative bacteria and *B. cereus*.

TCBS agar medium or BTB teepol agar medium has been used to identify *V. parahaemolyticus*. Horie *et al.*⁸⁾ developed one specific medium by using the arabinose-degrading ability of this species, and Baross *et al.*⁹⁾ and Twedt *et al.*¹⁰⁾ developed another medium by using the starch-degrading ability. These reports have been focused on selective isolation media, upon which bacterial samples were spread directly on agar plates. These methods require at least 24 hr to obtain results. On the other hand, the fluorogenic assay developed in the present study requires less time—about 8 hr, including the enrichment culture period, and *V. parahaemolyticus* populations over 400 cells/g were detectable (Fig. 3).

In the present method of *Vibrio* detection,

both the growth and trypsin-like activity of bacteria other than a few *Vibrio* species were suppressed in BSP-HP medium (Table 3). Since in this medium, *V. alginolyticus* and *V. harveyi*, as well as *V. parahaemolyticus*, grew vigorously and had high trypsin-like activity (Table 3), it was impossible to detect only *V. parahaemolyticus* specifically. However, *V. parahaemolyticus* infection is considered to be very frequent in foods with high trypsin-like activity. Therefore, the present method is useful as a rapid detection method for some *Vibrio* species including *V. parahaemolyticus*, for use in food quality control. The culture conditions and the specific medium that suppress *V. alginolyticus* and *V. harveyi* but enrich *V. parahaemolyticus* will be reported shortly.

Acknowledgments

The authors are deeply grateful to Prof. Y. Kuwabara, Kagawa Nutrition College, Prof. K. Shinagawa, Iwate University, Dr. H. Konuma, National Institute of Hygienic Science, Dr. M. Tokumaru, Saitama Institute of Public Health, Dr. S. Konno, Sendai Municipal Institute of Public Health and Dr. S. Umesako, Nara Prefec-

tural Institute of Public Health for their invaluable suggestions during the present investigation.

References

- 1) Nakashima, S., Takimoto, K.: Food Sanitation Research **37**(7), 50~76 (1987).
- 2) Pharmaceutical Society of Japan ed.: "Standard Methods of Analysis for Hygienic Chemists—with commentary—" p. 102~129 (1980), Kanehara Publishing Co., Tokyo.
- 3) Morichi, T.: Food Sanitation Research **34**(7), 23~38 (1984).
- 4) Maddocks, J. L., Greenan, M. J.: J. Clin. Pathol. **28**, 686~687 (1975).
- 5) Tsutsumi, M., Nishimura, K., Yasui, K., Matsuo, A., Watanabe, T.: J. Food Hyg. Soc. Japan **17**, 273~275 (1976).
- 6) Feng, P. C. S., Hartmann, P. A.: Appl. Environ. Microbiol. **43**, 1,320~1,329 (1982).
- 7) Moberg, L. J.: *ibid.* **50**, 1,383~1,387 (1985).
- 8) Horie, S., Yamada, Y., Tanaka, H., Yamashita, Y., Aihara, T.: J. Food Hyg. Soc. Japan **19**, 383~391 (1978).
- 9) Baross, J., Liston, J.: Appl. Microbiol. **20**, 179~186 (1970).
- 10) Twedt, R. M., Novelli, R. M. E.: *ibid.* **22**, 593~599 (1971).