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Improvement of the Immunomagnetic Separation Method Selective for *Escherichia coli* O157 Strains

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Immunomagnetic separation is a useful enrichment method selective for *Escherichia coli* O157 cells against non-O157 *E. coli* cells from a preenrichment culture. However, *E. coli* cells are adsorbed onto a solid surface nonspecifically. With the conventional immunomagnetic separation method, this nonspecific adsorption interfered with immunomagnetic separation. It was found that this interference could be reduced with a low-ionic-strength solution. When immunomagnetic separation was carried out with this solution, the proportion of *E. coli* O157 cells to non-O157 *E. coli* cells increased from 9.6 to 31.4 times compared to the proportion obtained by the conventional immunomagnetic separation method. The effectiveness of this solution was successfully evaluated by the use of *E. coli* O157-spiked samples.

Outbreaks of *Escherichia coli* O157 posed serious health threats in 1996 in Japan. Several mass infections in schools involving more than 6,000 patients and three deaths in Sakai City occurred, and school lunches were identified as the possible source of infection on the basis of epidemiological research (7). In almost all cases, the causative foods were not identified.

Conventional methods with selective enrichment broths for the detection and isolation of fecal coliforms in food samples require incubation at 44.5°C. However, *E. coli* O157:H7 cannot grow at this temperature, and as a result, these selective methods cannot be applied to differentiate this organism from most other nonpathogenic strains (8). Foods such as raw ground beef can be contaminated with a considerable number of coliforms (1,000 per g or more) (9) and can contain an average of six *E. coli* strains (12). The infectious dose of *E. coli* O157:H7 is believed to be very low (4). The recovery of the pathogen from the contaminated food might be hampered by the presence of large numbers of nonpathogenic *E. coli* strains in the enrichment culture for *E. coli.*

The effectiveness of the immunomagnetic separation (IMS) method with magnetic beads coated with antibodies against *E. coli* O157 as a selective enrichment method for *E. coli* O157 strains (1, 13), especially when the method is applied to fecal specimens (3, 5), has been well established. However, it was difficult to identify the vehicle of infection in most of the *E. coli* O157 outbreaks in Japan, suggesting that more sensitive methods are needed to isolate the small number of organisms present in suspected food.

It was known that *Vibrio parahaemolyticus* strains were adsorbed onto immunomagnetic beads nonspecifically and that the ability of adsorption differed among the strains. I established a method to prevent this nonspecific adsorption in the case of IMS selective for *V. parahaemolyticus* serotype K (10). In the IMS selective for *E. coli* O157 strains, non-O157 *E. coli* strains which contaminate food may also be present in large numbers in the enrichment culture and adsorb onto immunomagnetic beads nonspecifically, consequently reducing the sensitivity of the IMS method.

Initial bacterial adsorption to a solid surface was found to be affected by the presence of electrolytes in a suspending medium (6, 11). The bacteria were adsorbed in the presence of an electrolyte, and it was shown that a divalent electrolyte is more effective than a monovalent electrolyte. The adsorption decreased as the electrolyte concentration decreased. The mechanism of adsorption was interpreted in terms of the balance between the electrostatic repulsion forces and van der Waals attraction forces (2).

In this study, a method to prevent nonspecific adsorption of *E. coli* strains was devised and applied for the improvement of IMS sensitivity. For this purpose, the effect of electrolyte concentration on adsorption was investigated with low-ionic-strength water. Ultrapure water with a resistivity of 18.2 MΩ (Milli-Q [MQ] SP; Nihon Millipore Ltd.) was treated with cation-exchange resin to make low-ionic-strength water. Five grams of analytical grade Chelex 100 chelating ion-exchange resin (Bio-Rad Laboratories) was mixed with 100 ml of ultrapure water (MQ) and stood overnight at room temperature to chelate ions and settle the resin. The supernatant (chelex-treated MQ [CMQ]) was used as the low-ionic-strength solution. Brain heart infusion broth (BHI; Difco Laboratories) was treated in the same way with Chelex 100. *E. coli* could not grow or grew very poorly in this medium and could not grow in CMQ either. Whether or not the CMQ was sterilized did not affect the results, so CMQ was used without sterilization.

IMS is conventionally carried out by mixing Dynabeads (coated with polyclonal antibody against *E. coli* O157 [anti-E. coli O157]) (~10^8 beads/ml; Dynal A.S., Oslo, Norway) with a preenrichment culture of *E. coli*. The same experiment was carried out with CMQ. There were several possible choices for the preenrichment medium, but BHI was used in this experiment because it has no selectivity for *E. coli* growth. The degree of bacterial adsorption onto Dynabeads was compared with that of cells suspended in BHI and in CMQ. The following four non-O157 *E. coli* strains were employed to examine the effectiveness of the low-ionic-strength water on bacterial adsorption onto Dynabeads. *E. coli* strains V8 Nal^r, V9 Nal^r, V28 Nal^r, and V29 Nal^r were initially isolated from clinical specimens, and then nalidixic-acid-resistant spontaneous mutant strains were isolated from the colonies grown on nalidixic-acid (12.5 μg/ml)-supplemented MacConkey agar plates on which each *E. coli* strain was densely plated (0.5 ml of BHI overnight culture) and cultured 24 to 48 h at 37°C.

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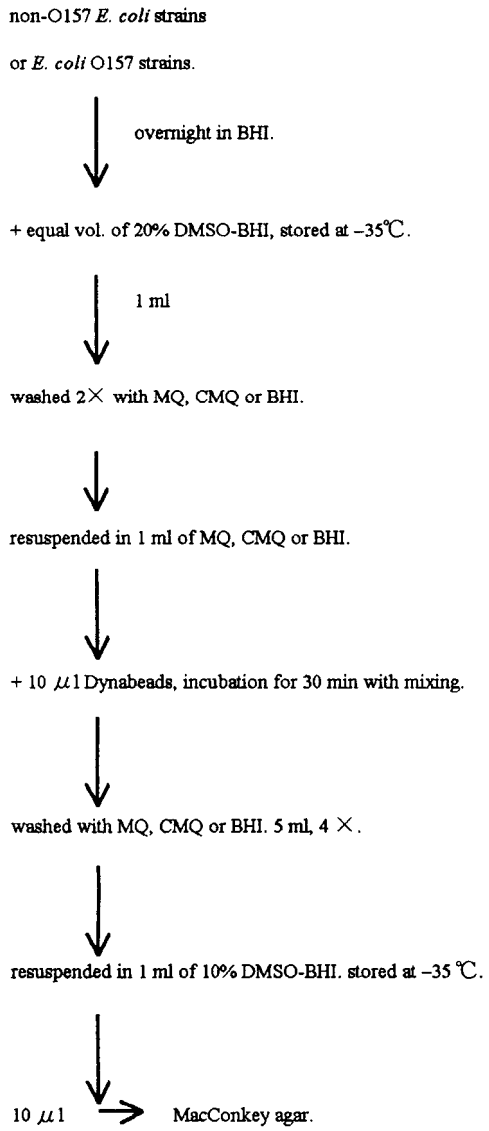

FIG. 1. Procedure for measurement of adsorption ability of *E. coli* onto Dynabeads.

The experimental design for bacterial adsorption is illustrated in Fig. 1. Cells were cultured overnight in BHI at 37°C. Each culture was mixed with an equal volume of 20% dimethyl sulfoxide (DMSO) (vol/vol) containing BHI (20% DMSO-BHI) and stored at −35°C until use. One milliliter of each culture was washed twice with MQ, CMQ, or BHI (as a control) by centrifugation (8 ml for 10 min at 3,500 rpm) and then resuspended in 1.0 ml of MQ, CMQ, or BHI, respectively. Polystyrene round-bottom tubes (16 by 125 mm; Falcon) were used. Ten microliters of Dynabeads was added to the suspension, and the suspension was incubated for 30 min at room temperature with occasional shaking. Five milliliters of MQ, CMQ, or BHI was added to the mixture. The nonadsorbed cells were separated from the Dynabead-adsorbed cells by placing the tube on a magnetic particle concentrator (model MPC-1; Dynal A.S.). The nonadsorbed cells were discarded, and the Dynabead-adsorbed cell fraction was washed four times by the same magnet procedure. The washed Dynabeads were resuspended in 1 ml of 10% DMSO-BHI and stored at −35°C. At the same time, 10 μl of these suspensions was plated onto MacConkey agar plates following serial 10-fold dilution in CMQ to find the appropriate dilution range. Colonies were counted after overnight incubation at 37°C. Then the stored suspension was thawed, and after appropriate dilution, the number of colonies in each suspension was determined (Table 1).

TABLE 1. Effect of solution upon adsorption of *E. coli* to Dynabeads (coated with anti-*E. coli* O157)

Strain	Cell concn in medium (cells/ml ± SD)	Solution used for adsorption and washing	No. of cells adsorbed (cells/ml ± SD)
V8 Nal^r	$(5.5 \pm 3.0) \times 10^7$	BHI	$(5.3 \pm 0.6) \times 10^3$
		MQ	$(7.5 \pm 0.7) \times 10^3$
		CMQ	$(6.4 \pm 4.8) \times 10^1$
V9 Nal^r	$(4.0 \pm 0.3) \times 10^8$	BHI	$(6.5 \pm 0.4) \times 10^6$
		MQ	$(3.3 \pm 0.2) \times 10^6$
		CMQ	$(4.2 \pm 0.1) \times 10^4$
V28 Nal^r	$(3.9 \pm 0.1) \times 10^8$	BHI	$(1.8 \pm 0.2) \times 10^4$
		MQ	$(1.8 \pm 0.3) \times 10^5$
		CMQ	$(2.6 \pm 0.5) \times 10^2$
V29 Nal^r	$(3.9 \pm 0.9) \times 10^8$	BHI	$(2.4 \pm 0.4) \times 10^4$
		MQ	$(5.5 \pm 0.4) \times 10^6$
		CMQ	$(2.0 \pm 0.9) \times 10^2$

These Dynabead suspensions contain more than 10^6 beads/ml. There is a possibility that more than one cell attached to a single bead, especially in the cases of V9 Nal^r (in BHI and in MQ) and V29 Nal^r (in MQ). However, the number of resultant colonies which were treated in CMQ was far lower than the number of beads in the plating sample. This result means that for CMQ, the possibility that more than one cell adsorbed onto a single bead was remote. Thus, the experiments were performed with the assumption that most of the colonies originated from a single cell attached to a single bead when the number of colonies was far lower than the number of beads.

Adsorption of non-O157 *E. coli* onto Dynabeads decreased by 1/69 (V28 Nal^r strain) to 1/828 times (V8 Nal^r strain) in CMQ compared to BHI. However, MQ was not effective and slightly stimulated adsorption, suggesting that the presence of the remaining electrolytes affected adsorption in MQ (Table

TABLE 2. Effect of solution upon adsorption of *E. coli* O157 strains to Dynabeads (M280; coated with sheep anti-rabbit IgG)

Strain	Cell concn in the medium (cells/ml ± SD)	Solution used for adsorption and washing	No. of cells adsorbed (cells/ml ± SD)
$O157_{KB0}$ Rif^r	$(2.7 \pm 1.1) \times 10^8$	BHI	$(6.8 \pm 3.1) \times 10^2$
		CMQ	$(1.6 \pm 0.3) \times 10^6$
$O157_{KB1}$ Rif^r	$(3.3 \pm 0.9) \times 10^7$	BHI	$(3.0 \pm 1.4) \times 10^2$
		CMQ	$(2.1 \pm 0.6) \times 10^5$
$O157_{KB2}$ Rif^r	$(5.8 \pm 1.6) \times 10^8$	BHI	$(2.0 \pm 0.3) \times 10^4$
		CMQ	$(7.4 \pm 1.1) \times 10^5$
$O157_{KB3}$ Rif^r	$(4.8 \pm 1.5) \times 10^8$	BHI	$(4.5 \pm 0.7) \times 10^2$
		CMQ	$(2.8 \pm 0.3) \times 10^6$
V9 Nal^r	$(5.5 \pm 0.3) \times 10^8$	BHI	$(1.3 \pm 0.1) \times 10^5$
		CMQ	$(3.7 \pm 0.5) \times 10^3$

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TABLE 3. Effect of solution upon adsorption of *E. coli* $O157_{KB0}$ Rif^r and V9 Nal^r in the mixed cell suspension to Dynabeads (M280; coated with sheep anti-rabbit IgG)

Before adsorption (cells/ml ± SD)			Solution used for:		No. of cells adsorbed (cells/ml ± SD)		
Rifampin selection	Nalidixic acid selection	Ratio of cells (Rif^r:Nal^r)	Adsorption	Washing	Rifampin selection	Nalidixic acid selection	Ratio of cells (Rif^r:Nal^r)
$(2.7 \pm 1.1) \times 10^5$	$(5.5 \pm 0.3) \times 10^8$	4.9×10^{-4}:1	BHI	BHI	5 ± 10	$(4.5 \pm 1.1) \times 10^4$	1.1×10^{-4}:1
			CMQ	CMQ	$(1.5 \pm 0.4) \times 10^3$	$(8.9 \pm 2.2) \times 10^2$	1.7:1

1). The ability of adsorption differed among the strains, but the reduction of adsorption in CMQ was remarkable among all strains.

Nonspecific adsorption of O157 strains onto beads in CMQ was also examined with Dynabeads (M280 sheep anti-rabbit immunoglobulin G [IgG]) (6×10^8 to 7×10^8 beads/ml), which is equivalent to Dynabeads (anti-E. coli O157) except for the coated antibody. The four *E. coli* O157:H7 strains used, $O157_{KB0}$ Rif^r, $O157_{KB1}$ Rif^r, $O157_{KB2}$ Rif^r, and $O157_{KB3}$ Rif^r, were spontaneous rifampin-resistant mutants from the collection of the Kobe Institute of Health. These mutants were isolated from the colonies on rifampin (100 μg/ml)-supplemented MacConkey agar plates in the same manner as the nalidixic-acid-resistant mutants. The $O157_{KB0}$ Rif^r strain was isolated from bovine stomachs, and other strains were isolated from hemorrhagic colitis patients in different years. Overnight BHI cultures of these strains were examined for adsorption onto Dynabeads the same way non-O157 strains were (Fig. 1). The V9 Nal^r strain was also employed as a control to examine the difference of adsorption between Dynabeads (M280 sheep anti-rabbit IgG) and Dynabeads (anti-E. coli O157). Unexpectedly, all these O157 strains were adsorbed onto the beads 37 times ($O157_{KB2}$ Rif^r strain) to 6,200 times ($O157_{KB3}$ Rif^r strain) more efficiently in CMQ than in BHI (Table 2). This result was confirmed by a mixed cell suspension. *E. coli* $O157_{KB0}$ cells were mixed with a 2,000-times-larger number of V9 Nal^r cells. The ratio of *E. coli* $O157_{KB0}$ Rif^r cells to V9 Nal^r cells was compared before and after adsorption onto the beads. After adsorption in CMQ, the adsorbed $O157_{KB0}$ Rif^r cell-to-V9 Nal^r cell ratio increased 3,500 times compared to the initial ratio in the mixed cell suspension (Table 3). It is not clear at present whether all O157 strains have the same characteristics and what the mechanism related to adsorption onto the beads is. However, this characteristic is beneficial for selecting O157 strains against non-O157 strains.

Accordingly, CMQ was applied to IMS (Fig. 2). The V9 Nal^r strain, which showed the highest adsorption ability among non-O157 *E. coli* (Table 1), was selected as a competitive background strain on the assumption that nonpathogenic strains were usually abundant in the *E. coli* enrichment culture. Four *E. coli* O157:H7 strains, $O157_{KB0}$ Rif^r, $O157_{KB1}$ Rif^r, $O157_{KB2}$ Rif^r, and $O157_{KB3}$ Rif^r, were employed. The *E. coli* O157 strains and the competitive V9 Nal^r strain were grown overnight in BHI. An equal volume of 20% DMSO-BHI was mixed and stored at −35°C until use. *E. coli* O157 cultures were thawed and diluted 1,000 times in BHI, and 1 ml of this diluted culture was mixed with 1 ml of thawed undiluted V9 Nal^r culture. This mixed cell suspension contained 10^4 to 10^5 *E. coli* O157 cells per ml and about a 10^3-times-larger number of V9 Nal^r cells (5.5×10^8 cells per ml). Two milliliters of the mixed cell suspension was washed twice with CMQ or BHI by centrifugation, and the pelleted cells were resuspended in 1 ml of CMQ or BHI, respectively. IMS was carried out conventionally with a preenrichment culture. In these experiments, BHI was used as the preenrichment medium. Then 10 μl of Dynabeads was added to each cell mixture and incubated for 30 min with occasional mixing. The nonbinding cells in the suspension in CMQ were washed with CMQ, and the nonbinding cells in the control BHI medium were washed with phosphate-buffered saline (PBS), pH 7.4, with Tween 20 (0.05% [vol/vol]) (PBS-

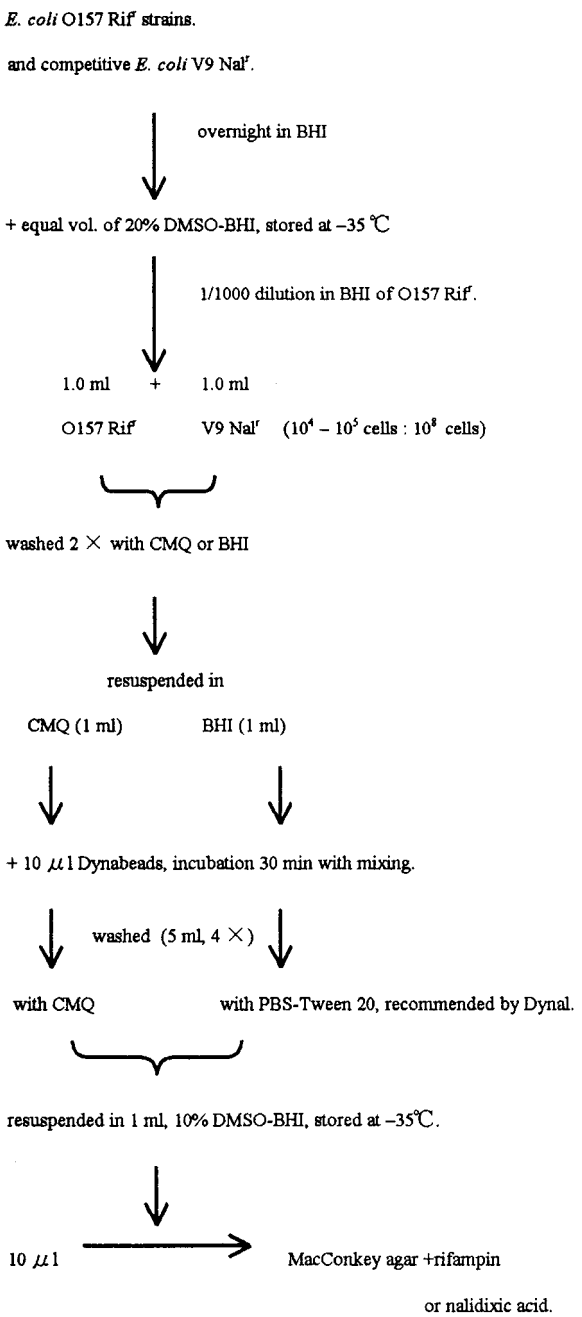

FIG. 2. Procedure for IMS selective for *E. coli* O157.

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TABLE 4. IMS of *E. coli* O157 cells from a large number of competitive V9 Nal^r cells

E. coli O157 strain selected	Before IMS			Solution used for:		After IMS		
	Cells/ml ± SD					Cells/ml ± SD		
	Rifampin selection	Nalidixic acid selection	Ratio of cells (Rif^r:Nal^r)	IMS	Washing	Rifampin selection	Nalidixic acid selection	Ratio of cells (Rif^r:Nal^r)
O157$_{KB0}$ Rif^r	$(2.7 \pm 1.1) \times 10^5$	$(5.5 \pm 0.3) \times 10^8$	$1:2.0 \times 10^3$	BHI	PBS-Tween 20	$(2.7 \pm 0.4) \times 10^4$	$(6.6 \pm 1.0) \times 10^3$	4.1:1
				CMQ	CMQ	$(2.9 \pm 1.0) \times 10^3$	$(7.2 \pm 5.4) \times 10^1$	40:1
O157$_{KB1}$ Rif^r	$(3.3 \pm 0.9) \times 10^4$	$(5.5 \pm 0.3) \times 10^8$	$1:1.7 \times 10^4$	BHI	PBS-Tween 20	$(1.4 \pm 0.2) \times 10^4$	$(7.7 \pm 0.8) \times 10^3$	1.8:1
				CMQ	CMQ	$(1.3 \pm 0.1) \times 10^4$	$(2.3 \pm 0.9) \times 10^2$	56.5:1
O157$_{KB2}$ Rif^r	$(5.8 \pm 1.6) \times 10^5$	$(5.5 \pm 0.3) \times 10^8$	$1:9.5 \times 10^2$	BHI	PBS-Tween 20	$(2.9 \pm 1.0) \times 10^4$	$(5.1 \pm 0.6) \times 10^3$	5.7:1
				CMQ	CMQ	$(9.3 \pm 3.8) \times 10^3$	$(1.7 \pm 0.5) \times 10^2$	54.7:1
O157$_{KB3}$ Rif^r	$(4.8 \pm 1.5) \times 10^5$	$(5.5 \pm 0.3) \times 10^8$	$1:1.1 \times 10^3$	BHI	PBS-Tween 20	$(3.2 \pm 1.2) \times 10^4$	$(3.4 \pm 0.8) \times 10^3$	9.4:1
				CMQ	CMQ	$(5.7 \pm 1.2) \times 10^4$	$(2.0 \pm 0.8) \times 10^2$	285:1

Tween 20) the same way as in the adsorption experiments (5 ml, four times). The efficacy of washing with CMQ was compared with that of washing with PBS-Tween 20 because the manufacturer (Dynal) recommended the latter.

After being washed, the cells were resuspended in 1 ml of 10% DMSO-BHI and stored at −35°C. Then an appropriate preliminary dilution range for Rif^r and Nal^r cells was determined with a portion of these suspensions. Ten microliters was plated onto a MacConkey agar plate supplemented with rifampin (100 μg/ml) or nalidixic acid (12.5 μg/ml). Following overnight incubation, the magnitude of the enrichment of *E. coli* O157 Rif^r strains against the competitive V9 Nal^r strain was measured by comparing the number of Rif^r colonies and Nal^r colonies that resulted (Table 4).

The initial proportion of Rif^r cells to Nal^r cells in the mixed cell suspension ($1:9.5 \times 10^2$ to $1:1.7 \times 10^4$) increased by 5.4×10^3 to 3.0×10^4 times after IMS when the conventional IMS method was applied.

When IMS was carried out in and washed away by CMQ, the proportion of Rif^r cells to Nal^r cells increased 9.6 (O157$_{KB2}$ Rif^r) to 31.4 (O157$_{KB1}$ Rif^r) times compared to the proportion obtained by the conventional IMS method. In conventional routine work, several colonies which look like *E. coli* on the MacConkey agar plate are directly examined with an *E. coli* O157 latex test kit (Oxoid) or isolated and inoculated in an appropriate medium such as triple sugar iron agar and sulfide indole motility medium for further biotyping and serotyping for identification of *E. coli* O157. It is essential to examine a large number of colonies to find O157 colonies that are present in small numbers. The results obtained for IMS which was carried out in and washed away by CMQ indicate that the chances of finding O157 colonies are 9 to 31 times better than for conventional methods. This improved IMS would help isolate *E. coli* O157 strains which may be present in food or environmental samples in small numbers.

Isolation of *E. coli* O157 from artificially inoculated minced beef was carried out to verify the effectiveness of CMQ. Five samples of minced beef were purchased from five different retailers, and equal weights of these samples were mixed well. Portions of 10 g were stored until use at −35°C in a sterile stomacher bag which was recommended as a preenrichment culture container by Dynal. Ten grams of the sample was mixed with 90 ml of buffered peptone water (BPW; Oxoid), which was also recommended by Dynal as a preenrichment medium for food samples, and then mixed in the stomacher. After serial dilution with saline, the total number of viable bacterial cells was counted by plating onto a plate count agar, and the number of coliform bacteria was estimated by plating onto a desoxycholate agar plate (Table 5). Species identification was made for nine coliform colonies on the plate of the highest dilution (ID test EB-20 for the identification of lactose-fermenting, gram-negative, rod-shaped bacteria; Nissui). Eight colonies were *Enterobacter* spp., and one colony was *Klebsiella* spp. MacConkey agar was used exclusively to isolate *E. coli* in these experiments. These colonies, which were grown on MacConkey agar plates, were difficult to distinguish from *E. coli* colonies. Colony counts were also performed on MacConkey agar plates and on nalidixic-acid-supplemented and rifampin-supplemented MacConkey agar plates. MacConkey agar suppressed the growth of some bacterial species. There were fewer viable colonies on this plate than on the plate count agar. The number of lactose-fermenting (red-colored) colonies grown on MacConkey agar plates was about the same as that of the coliform colonies on the desoxycholate agar plates. No lactose-fermenting colonies in 0.02 g of the sample grew on rifampin- or nalidixic-acid-supplemented MacConkey agar plates. These observations indicated that there was no rifampin- or nalidixic-acid-resistant coliform in this minced-beef sample.

For comparison of the relative effectiveness of the IMS technique with the CMQ treatment and the conventional method, *E. coli* O157 cells were inoculated along with non-O157 *E. coli* Nal^r cells to measure the degree of improvement (Fig. 3). Ten-gram portions of the minced beef were mixed with 90 ml

TABLE 5. Viable cell count in minced beef

Medium and cell type	Cells/g ± SD
Plate count agar	
Total viable cells	$(1.8 \pm 0.1) \times 10^6$
Desoxycholate agar	
Coliform cells	$(5.1 \pm 0.1) \times 10^3$
MacConkey agar	
Total	$(6.2 \pm 0.2) \times 10^4$
Lactose fermenting	$(7.0 \pm 2.9) \times 10^3$
Rifampin-supplemented MacConkey agar	
Lactose fermenting	0[a]
Lactose nonfermenting	$(1.7 \pm 0.5) \times 10^2$
Nalidixic acid supplemented MacConkey agar	
Lactose fermenting	0[a]
Lactose nonfermenting	$(2.5 \pm 0.5) \times 10^3$

[a] 0 indicates no cells in 0.02 g of the minced beef.

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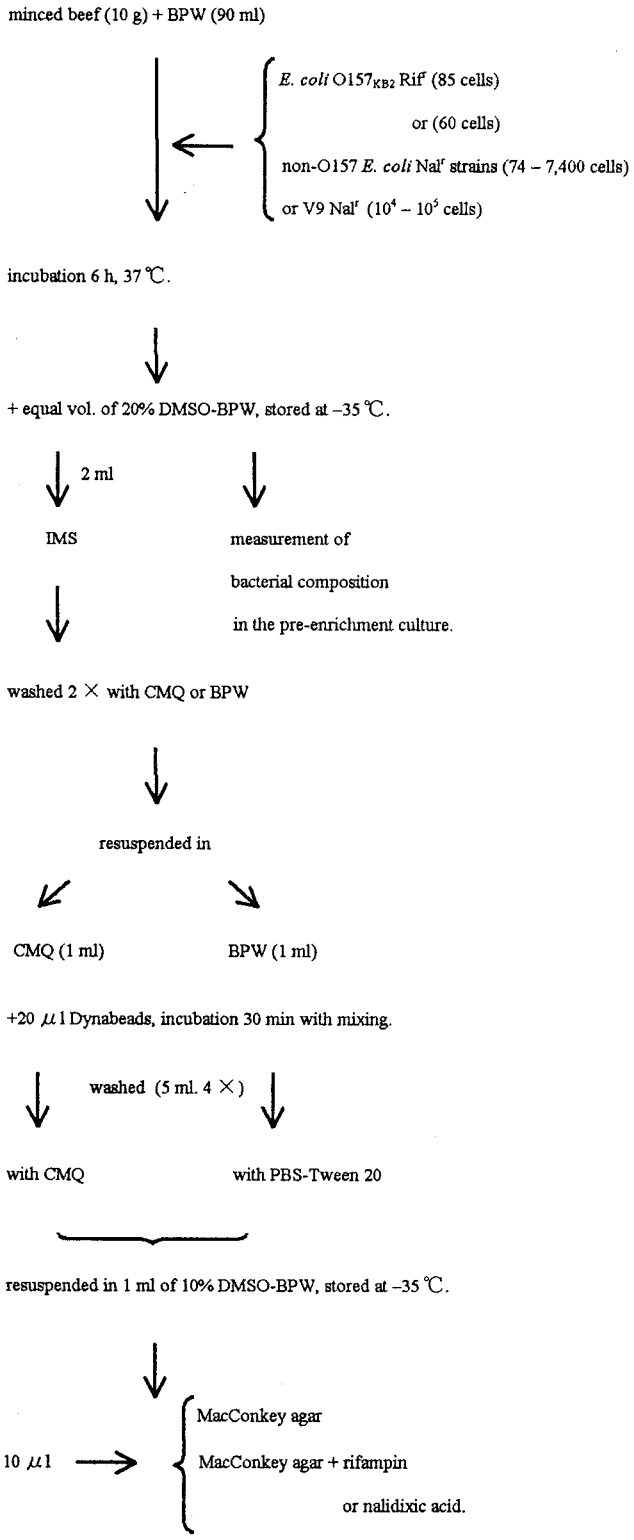

FIG. 3. Procedure for isolation of *E. coli* O157 from the spiked minced beef.

of BPW. A small number of *E. coli* $O157_{KB1}$ Rif^r cells (85) and a different number of competitive non-O157 *E. coli* Nal^r cells (74 to 7,400) were inoculated into these mixtures and mixed briefly in the stomacher. Non-O157 *E. coli* Nal^r cells were a

TABLE 6. Recovery of *E. coli* O157 strain from the spiked minced beef by IMS

Expt no.	No. of *E. coli* cells inoculated		Preenrichment culture				Solution used for:		After IMS			
			MacConkey agar (cells/ml ± SD)						MacConkey agar (cells/ml ± SD)			
	$O157_{KB2}$ Rif^r	Non-O157 *E. coli* cells[b]	Total cells	Rifampin selection	Nalidixic acid selection	Ratio of cells (Rif^r:Nal^r)	IMS	Washing	Total cells	Rifampin selection	Nalidixic acid selection	Ratio of cells (Rif^r:Nal^r)
A	85 ± 28	74 ± 3	(1.9 ± 0.4) × 10^7	(4.8 ± 1.4) × 10^5	(3.0 ± 0.8) × 10^5	1.6:1	BPW	PBS-Tween 20	(4.2 ± 0.1) × 10^5	(5.4 ± 1.4) × 10^4	(1.0 ± 0.1) × 10^3	5.4 × 10^1:1
							CMQ	CMQ	(1.6 ± 0.2) × 10^4	(5.6 ± 1.3) × 10^3	0[a]	>5.6 × 10^3:1
B	85 ± 28	(74 ± 3) × 10^1	(3.8 ± 0.2) × 10^7	(4.4 ± 0.6) × 10^4	(2.6 ± 0.2) × 10^5	1.7 × 10^{-1}:1	BPW	PBS-Tween 20	(1.4 ± 0.4) × 10^5	(7.6 ± 1.5) × 10^3	(9.4 ± 2.3) × 10^2	1.8 × 10^1:1
							CMQ	CMQ	(1.1 ± 0.1) × 10^4	(1.6 ± 0.3) × 10^3	5 ± 10	3.2 × 10^2:1
C	85 ± 28	(74 ± 3) × 10^2	(2.2 ± 0.2) × 10^7	(8.0 ± 1.0) × 10^4	(3.4 ± 0.4) × 10^6	2.5 × 10^{-2}:1	BPW	PBS-Tween 20	(8.1 ± 1.1) × 10^4	(1.2 ± 0.04) × 10^4	(6.8 ± 0.3) × 10^3	1.7:1
							CMQ	CMQ	(6.3 ± 0.7) × 10^3	(2.7 ± 1.0) × 10^2	0[a]	>2.7 × 10^2:1
D	60 ± 2	(97 ± 2) × 10^2	(4.0 ± 0.6) × 10^7	(9.0 ± 4.6) × 10^4	(1.8 ± 0.4) × 10^7	5.1 × 10^{-3}:1	BPW	PBS-Tween 20	(2.2 ± 0.4) × 10^5	(5.4 ± 1.0) × 10^4	(4.2 ± 0.3) × 10^4	1.3:1
							CMQ	CMQ	(4.3 ± 0.6) × 10^3	(3.2 ± 0.7) × 10^2	5 ± 10	6.4 × 10^1:1
E	60 ± 2	(97 ± 2) × 10^3	(9.4 ± 0.6) × 10^7	(4.6 ± 1.0) × 10^4	(1.1 ± 0.1) × 10^8	4.3 × 10^{-4}:1	BPW	PBS-Tween 20	(4.2 ± 0.4) × 10^5	(1.9 ± 0.1) × 10^4	(3.7 ± 0.02) × 10^5	5.0 × 10^{-2}:1
							CMQ	CMQ	(7.3 ± 0.3) × 10^3	(2.1 ± 0.1) × 10^3	(3.1 ± 0.9) × 10^2	6.8:1
F	0	0	ND[c]	ND[c]	ND[c]	ND[c]	BPW	PBS-Tween 20	(8.5 ± 0.9) × 10^4	0[a]	0[a]	

[a] There were no cells in the 0.2-ml cell suspension.
[b] In experiments D through F, the non-O157 *E. coli* strain used was V9 Nal^r.
[c] ND, not determined.

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TABLE 7. Serotype examination of the *E. coli*-like colonies[a] grown on MacConkey agar plate after IMS

Expt no.	Solution used for:		No. of O157 antigen-positive and rifampin-resistant colonies	No. of O157 antigen-negative colonies		Recovered O157 colonies (%)
	IMS	Washing		Nalidixic acid resistant	Rifampin sensitive	
A	BPW	PBS-Tween 20	15	2	8	60
	CMQ	CMQ	23	0	2	92
B	BPW	PBS-Tween 20	20	1	4	80
	CMQ	CMQ	24	0	1	96
C	BPW	PBS-Tween 20	8	11	6[b]	32
	CMQ	CMQ	25	0	0	100
D	BPW	PBS-Tween 20	14	11	0	56
	CMQ	CMQ	24	1	0	96
E	BPW	PBS-Tween 20	3	22	0	12
	CMQ	CMQ	22	3	0	88

[a] A total of 25 colonies were examined.
[b] All six colonies were *H. alvei*.

mixture of equal volumes of overnight BHI culture of V8 Nal^r, V9 Nal^r, V28 Nal^r, and V29 Nal^r strains. Preenrichment cultures were performed at 37°C for 6 h. These cultures were mixed with an equal volume of 20% DMSO containing BPW (20% DMSO-BPW) and stored at −35°C until use. The number of colonies was counted, and the composition of each of the Rif^r and Nal^r *E. coli* colonies in the preenrichment culture was examined with a portion of frozen culture (Table 6). Since this minced beef contained no lactose-fermenting rifampin-resistant or nalidixic-acid-resistant cells, only *E. coli* Rif^r and Nal^r grew as red-colored colonies on rifampin- or nalidixic-acid-supplemented MacConkey agar plates. The small number of lactose-nonfermenting cells which grew as translucent colonies did not interfere with the counting of *E. coli* colonies.

These frozen cultures were applied to IMS selective for *E. coli* O157 strains. Two milliliters of the frozen culture was thawed and washed twice with CMQ or BPW by centrifugation. The cell pellets were resuspended in 1 ml of CMQ or BPW, respectively. By the Dynal method, IMS was carried out with a preenrichment BPW culture. Accordingly, BPW was adopted as the solution for IMS, and 20 μl of Dynabeads was added to the preenrichment culture. This volume of Dynabeads was as recommended in the Dynal protocol.

CMQ was also effective when applied to minced-beef samples (Table 6). By the Dynal method, the number of recovered $O157_{KB2}$ Rif^r strains decreased as the competitive *E. coli* Nal^r strains increased (the Rif^r-to-Nal^r ratio decreased from 54:1 [Table 6, experiment A] to 1.7:1 [Table 6, experiment C]). However, when the CMQ solution was applied, nonspecifically adsorbed *E. coli* Nal^r colonies were negligible.

The V9 Nal^r strain was highly adsorptive onto Dynabeads. This strain was adopted as the more tenacious competitive *E. coli* strain for the spiked experiment. Again in this case, *E. coli* $O157_{KB2}$ Rif^r strain could be isolated even when the number of inoculated V9 Nal^r cells was more than 1,000 times larger (Table 6, experiment E). These results were reconfirmed by examining 25 colonies on a MacConkey agar plate after IMS (Table 7). These colonies were selected by comparison with $O157_{KB2}$ Rif^r colonies to avoid any preference for the appearance of a colony. The presence of O157 antigens was determined by a slide agglutination test with an *E. coli* O157 latex test kit. The same colonies were inoculated onto nalidixic-acid- and rifampin-supplemented MacConkey agar plates to confirm resistance to these drugs. The ratio of recovered O157 strains to non-O157 coliforms was similar to the Rif^r-to-Nal^r colony ratio after IMS. Six O157 antigen-negative and rifampin-sensitive colonies (Table 7, experiment C) were selected as representatives of naturally contaminated coliform bacteria in these experiments and were identified as *Hafnia alvei.*

The absence of naturally contaminated verocytotoxin-producing *E. coli* O157 strains in these minced-beef samples was confirmed by PCR assay. The preenrichment culture of minced beef, in which there were no inoculated *E. coli* strains, was used for IMS. The recovered beads were assayed by the PCR method for the detection of verocytotoxin genes. The number of beads examined was equivalent to 0.5 ml of bead suspension after IMS and contained 4.3×10^4 cells (Table 6, experiment F). No verocytotoxin gene was detected in this sample, while 0.5 ml of equivalent beads in the experiment (Table 6, experiment B: BPW used for IMS and PBS-Tween 20 used for washing; 7×10^4 cells), which was adopted as the positive control, showed the presence of VT1 and VT2 genes (data not shown).

CMQ proved to be efficient to prevent nonspecific adsorption of other non-O157 *E. coli* cells onto Dynabeads. This improved IMS method is applicable for the isolation of many other pathogenic bacteria for which antibodies are available.

I previously described a method for selective enrichment for *Vibrio parahaemolyticus* serotype K, using a rabbit antiserum kit for *V. parahaemolyticus* and Dynabeads (M280 sheep anti-rabbit IgG) (10). However, an attempt to select a specified serotype of *V. parahaemolyticus* was not successful by this method because halophilic *V. parahaemolyticus* cells lysed immediately after exposure to CMQ. The bacteria selected for this test must be hardy enough to withstand low-ionic-strength solutions.

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