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An Improved Enrichment Broth for Isolation of *Escherichia coli* O157, with Specific Reference to Starved Cells, from Radish Sprouts

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An enrichment broth was developed for the efficient isolation of *Escherichia coli* O157 from radish sprouts. The broth was buffered peptone water containing 0.5% sodium thioglycolate (STG-BPW), which was designed to allow growth of *E. coli* O157 in starved and unstarved states. However, this medium suppressed the growth of non-carbohydrate-fermenting obligate aerobes whose colonial appearance on sorbitol MacConkey agar containing cefixime and tellurite (CT-SMAC) resembled that of *E. coli* O157. Both starved and unstarved cells of *E. coli* O157 experimentally inoculated into radish sprouts were successfully recovered with STG-BPW enrichment in all cases, most of which showed marked disappearance of *E. coli* O157-like colonies on CT-SMAC.

Enterohemorrhagic *Escherichia coli* O157 has been increasingly recognized as a major food-borne pathogen that causes hemorrhagic colitis and hemolytic-uremic syndrome (10, 12). Beef and dairy cattle are considered to be natural reservoirs of this pathogen (3), and thus, most infections have been associated with consumption of undercooked ground beef or raw milk (6, 7). Although occurring less frequently, outbreaks caused by consumption of raw vegetables contaminated with *E. coli* O157 have also been reported in various parts of the world (2, 19). In 1996, a large outbreak, involving more than 6,000 primary schoolchildren, occurred in Sakai City, Osaka Prefecture, Japan. An epidemiological investigation (14) revealed that raw radish (*Raphanus sativus*) sprouts served in school lunches were the most likely cause; the radish sprouts were grown hydroponically and could have been contaminated with *E. coli* O157 during cultivation. However, viable *E. coli* O157 cells were seldom detected in samples taken from the radish sprouts in question.

Despite these incidences of infection, radish sprouts are still commercially available in Japan, being frequently consumed by the public. It is therefore of paramount importance to establish an appropriate method by which to detect possible *E. coli* O157 contamination of this particular food item. In this connection, we have demonstrated that enrichment cultures using a selective medium and/or at a high temperature are unsuitable for the isolation of *E. coli* O157 from water samples (16). More recently, Fujisawa et al. have reported that many bacteria that formed colorless colonies similar to those of *E. coli* O157 on sorbitol MacConkey agar containing cefixime and tellurite (CT-SMAC) were present in radish sprouts, causing difficulty in selecting *E. coli* O157 colonies on the plate (9). Here we describe a novel enrichment culture method that is designed to facilitate the growth of both starved and unstarved cells of *E. coli* O157 but suppress the growth of concomitant *E. coli* O157-like colonies on CT-SMAC for successful isolation of *E. coli* O157 from radish sprouts.

The bacterial strains used included 19 strains of Shiga toxin-producing *E. coli* O157:H7 or NM from various sources such as patient or cattle feces and foods and 15 strains of non-*E. coli* O157 bacteria that had been isolated from commercially available radish sprouts during our routine practice. These non-*E. coli* O157 bacteria were all gram-negative rods that formed colorless or slightly pinkish colonies on CT-SMAC plates, some remarkably resembling those of *E. coli* O157. Of these 15 strains, 5 were glucose fermenting but not sorbitol fermenting and 10 were non-carbohydrate fermenting. Four of these non-sorbitol-fermenting strains were identified by commercially available identification kits (API 20E and API 20NE; API System, Montalieu-Vercieux, France) as *Enterobacter cloacae*, *Aeromonas hydrophila*, or *Hafnia alvei*. Five of the non-carbohydrate-fermenting strains were identified as *Pseudomonas aeruginosa*, *Alcaligenes xylosoxidans*, *Ralstonia pickettii*, or *Acinetobacter lwoffii*, but the identities of the other five strains could not be determined.

We prepared two different enrichment media: (i) buffered peptone water (BPW; Oxoid, Basingstoke, England) and (ii) BPW supplemented with 0.5% sodium thioglycolate (STG; Wako Pure Chemical Co. Ltd., Osaka, Japan) (BPW-STG). It should be noted that STG has a reducing process, thereby making the liquid medium anaerobic and hence unsuitable for the growth of aerobes (15). For studies of growth on artificially contaminated radish sprouts, we used BPW, STG-BPW, and modified EC broth (Eiken Chemical Co., Ltd., Tokyo, Japan) containing novobiocin (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 20 mg/liter (mEC+n).

Starved and unstarved cells of *E. coli* O157 were prepared as described previously (9). Briefly, test strains that had been cultured at 37°C for 18 h in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) were washed with steril-

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TABLE 1. Rates of *E. coli* O157 recovery[a]

State of contaminating *E. coli* O157 cells and assay result	No. of samples							
	Aerobic culture (18 h)				Anaerobic culture (18 h)			
	36°C			42°C, mEC + n	36°C			42°C, mEC + n
	BPW	STG-BPW	mEC + n		BPW	STG-BP	mEC + n	
Unstarved								
High recovery	0	1	0	3	0	2	0	3
False negative	0	0	2	1	0	0	1	1
Starved								
High recovery	0	5	0	2	0	0	0	1
False negative	0	0	3	3	0	0	3	4

[a] Shown are the number of cases that showed a maximum rate of recovery of *E. coli* O157 colonies with a minimum rate of recovery of other colorless colonies on CT-SMAC plates (high recovery) and the number of cases in which *E. coli* O157 was not isolated (false negative) from six samples of radish sprouts experimentally contaminated with starved or unstarved *E. coli* O157 cells.

ized deionized water and resuspended in sterilized deionized water. Bacterial cells immediately after suspension and those kept in the dark at 23°C over a 3-week period were considered unstarved and starved cells, respectively.

First, an appropriately diluted suspension (0.1 ml) containing 1.1×10^3 to 2.6×10^3 starved or unstarved cells of *E. coli* O157 and that containing 1.0×10^3 to 6.6×10^3 unstarved cells of the non-sorbitol- and the non-carbohydrate-fermenting strains were each inoculated into 10 ml of BPW and STG-BPW and incubated aerobically or anaerobically in jars using Anaeropack Kenki (Mitsubishi Gas Chemicals Co. Ltd., Tokyo, Japan) at 36°C for 18 h. After incubation, each culture of *E. coli* O157 and the other strains was diluted with sterile saline and 0.1-ml volumes of serial 10-fold dilutions onto duplicate plates of heart infusion agar (Eiken) and then incubated at 36°C for 24 h. After incubation, the colonies on the agar plates were counted to evaluate growth. Regardless of the culture conditions, the growth of both starved (1.8×10^8 to 3.8 CFU/ml) and unstarved (1.8×10^8 to 4.0 CFU/ml) cells of all *E. coli* O157 isolates was comparable. All of the non-carbohydrate-fermenting strains had much less growth when incubated anaerobically or in the presence of STG since most are obligate aerobes. Only one of the non-sorbitol-fermenting strains showed such suppressed growth when incubated anaerobically or in the presence of STG.

Second, 3 g of radish sprouts and 0.1 ml of a bacterial suspension containing 1.3×10^3 to 3.2×10^3 starved or 0.5×10^3 to 1.7×10^3 unstarved cells of six randomly selected *E. coli* O157 isolates were added to 27 ml of BPW, STG-BPW, or mEC+n in sterilized screw-cap centrifuge tubes in which the initial concentration of *E. coli* O157 cells was adjusted to approximately 10 to 100 CFU/ml of broth medium. The experimentally contaminated radish sprouts thus prepared were incubated aerobically or anaerobically at 36 or 42°C for 18 h. It should be noted that a preliminary microbiological test confirmed the original radish sprouts as negative for *E. coli* O157 contamination. After incubation, 0.1-ml volumes of serial 10-fold dilutions of the spent medium were spread onto CT-SMAC agar plates (sorbitol MacConkey agar no. 3 [Oxoid] containing a solution of cefixime and tellurite [Selectivial; Mast Group Ltd., Merseyside, United Kingdom]). After incubation at 36°C for 20 to 22 h, the number of colonies that grew on the plates was determined. For differentiation of *E. coli* O157 colonies from others, colorless or slightly pinkish colonies on CT-SMAC were picked up and then tested by a commercial latex agglutination test kit (UNI; Oxoid) in order to determine whether they were *E. coli* O157 or not.

The number of cases that showed a maximum rate of recovery (40 to 100%) of *E. coli* O157 colonies from the total number of colonies grown on CT-SMAC and a minimum rate of recovery (0 to 7%) of other colorless colonies (high recovery) and the number of cases in which *E. coli* O157 was not isolated from six experimentally contaminated radish sprouts (false negative) are summarized in Table 1. For the radish sprouts contaminated with unstarved *E. coli* O157 cells, both aerobic and anaerobic enrichments with mEC+n at 42°C showed high recovery in three out of six cases but failed to isolate any *E. coli* O157 in one or two cases, yielding false-negative results. For the radish sprouts with the starved cells, aerobic enrichments with STG-BPW at 36°C showed high recovery of *E. coli* O157 in five out of the six cases whereas both aerobic and anaerobic enrichments with mEC+n at 36 or 42°C failed to isolate *E. coli* O157 in three or four cases.

Over the past decade, a number of *E. coli* O157 infections associated with water systems have been reported worldwide (1, 4, 8, 11, 13, 17). Horticultural vegetables such as radish sprouts are therefore considered to be under a great risk of contamination with water that contains viable *E. coli* O157 cells. As demonstrated elsewhere (5, 18), the cells in the water systems are most likely to be exposed to various physical, chemical, and nutritional stresses, with the majority being injured to some degree. In this connection, we have demonstrated that growth of *E. coli* O157 starved in sterile deionized water or filter-sterilized natural river water was markedly suppressed in mEC+n (16). This was consistent with the results of the present study. Unlike mEC+n, enrichment with STG-BPW will markedly improve the efficiency of isolation of starved *E. coli* O157 from radish sprouts, thereby preventing many testing laboratories from issuing false-negative results. The method may be used for detection of *E. coli* O157 in other vegetables and fruits that are likely to be contaminated with starved cells.

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