

Modification of Sorbitol MacConkey Medium Containing Cefixime and Tellurite for Isolation of *Escherichia coli* O157:H7 from Radish Sprouts

TOMOHIKO FUJISAWA,^{1*} SHIN SATA,¹ KATSUHIRO AIKAWA,¹ TAKANORI TAKAHASHI,¹
SHIRO YAMAI,¹ AND TOSHIO SHIMADA²

Kanagawa Prefectural Public Health Laboratory, Yokohama 241-0815,¹ and National Institute of Infectious Diseases, Shinjuku-ku, Tokyo 162-8640,² Japan

Received 22 December 1999/Accepted 13 April 2000

A modified version of sorbitol MacConkey medium containing cefixime and tellurite (CT-SMAC medium) was produced by adding salicin and 4-methylumbelliferyl- β -D-galactopyranoside to CT-SMAC medium; this medium was designated CT-SSMAC medium and was used to isolate *Escherichia coli* O157:H7 from radish sprouts. Of 101 non-*E. coli* bacteria isolated from radish sprouts that produced colorless colonies similar to colonies of *E. coli* O157:H7 grown on CT-SMAC medium, 92 (91%) formed colonies that were red to pink or were β -galactosidase negative and colorless on CT-SSMAC medium. On the other hand, colonies of *E. coli* O157:H7 strains were colorless and β -galactosidase positive on CT-SSMAC medium. Our results suggest that CT-SSMAC medium is more selective than CT-SMAC medium for isolating *E. coli* O157:H7.

Sorbitol MacConkey medium (SMAC medium) (11) and sorbitol MacConkey medium containing cefixime and tellurite (CT-SMAC medium) (1, 14) were described as media that can be used for isolation of *Escherichia coli* O157. Recently, an optimized method for detecting verocytotoxigenic *E. coli* in food by using CT-SMAC medium was introduced. However, it seems that modification of CT-SMAC medium is necessary to isolate *E. coli* O157:H7 from radish (*Raphanus sativus*) sprouts. During a study of the occurrence of *E. coli* O157:H7 in radish sprouts grown hydroponically, many colorless colonies similar to *E. coli* O157:H7 colonies, which do not produce acid from sorbitol, grew on CT-SMAC medium. Sata et al. (13) found that using modified *E. coli* broth supplemented with novobiocin or modified Trypticase soy broth supplemented with novobiocin as a liquid enrichment medium for *E. coli* O157:H7 is not suitable for isolating injured *E. coli* O157:H7 cells from water systems. Therefore, these authors recommended that a nonselective liquid enrichment medium should be used for isolation of *E. coli* O157:H7 (13). From these viewpoints, the role of selective agar plates in isolating *E. coli* O157:H7 is very important. In this study, we investigated using a modification of CT-SMAC medium for isolation of *E. coli* O157:H7 from radish sprouts.

The strains of *E. coli* O157:H7 and O157:NM (nonmotile) used in this study are listed in Table 1. Five clinical isolates from patients, six bovine fecal isolates, and eight food or environmental isolates were used. Nine strains (NIID 2, NIID 457, NIID 23, NIID 42, NIID 437, NIID 1124, NIID 1856, NIID 1646, and NIID 1496) were provided by H. Watanabe (National Institute of Infectious Diseases, Tokyo, Japan). Details concerning the *E. coli* O157:H7 and *E. coli* O157:NM strains have been described previously (13). The 101 gram-negative aerobic and facultatively anaerobic rod-shaped strains that were not *E. coli* strains were isolated from radish sprouts in our laboratory (Table 2). Ten lots of radish sprouts marketed in Japan were used. Samples (25 g) were incubated in 225 ml of buffered peptone water (Oxoid, Basingstoke, Hampshire, England) at 36°C for 18 h. After incu-

bation, one loopful of each culture was spread onto CT-SMAC medium and incubated at 36°C for 22 h. After this, colorless colonies that grew on the agar medium were picked and investigated to determine their oxidase activities. For oxidase-negative strains, we performed the indole test with SIM medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), the citrate utilization test with Simmons citrate agar (Nissui), and the methyl red and Voges-Proskauer tests with VP-MR medium (Nissui). Moreover, some isolates were identified to the species level by using the API 20E system (bioMérieux SA, Marcy l'Etoile, France).

A 0.05 g portion of 4-methylumbelliferyl- β -D-galactopyranoside (Wako Pure Chemical Industries Ltd., Osaka, Japan) was added to 495 ml of distilled water, and the preparation was gently sonicated to produce tiny particles. After this, 5 g of D-sorbitol (Difco Laboratories, Detroit, Mich.), 5 g of salicin (Difco), and 20 g of MacConkey agar base (Difco) were added,

TABLE 1. *E. coli* O157:H7 and O157:NM strains tested

Strain ^a	Source	Serotype
NIID 2	Patient feces	O157:H7
NIID 457	Implicated food	O157:H7
NIID 23	Patient feces	O157:H7
NIID 42	Patient feces	O157:H7
NIID 437	Patient feces	O157:H7
NIID 1124	Patient feces	O157:H7
NIID 1856	Implicated food	O157:H7
NIID 1646	Implicated food	O157:H7
NIID 1496	Fly	O157:H7
KE-10	Implicated food	O157:H7
KE-24	Sewage water	O157:H7
KE-31	Cattle feces	O157:H7
KE-87	Swab from a sink	O157:NM
KE-114	Food	O157:H7
KE-149	Cattle feces	O157:H7
KE-181	Cattle feces	O157:H7
KE-188	Cattle feces	O157:NM
KE-302	Cattle feces	O157:H7
KE-373	Cattle feces	O157:H7

^a NIID, National Institute of Infectious Diseases, Tokyo, Japan; KE, our collection.

* Corresponding author. Mailing address: Kanagawa Prefectural Public Health Laboratory, 1-1-1, Nakao, Asahi-ku, Yokohama, 241-0815, Japan. Phone: 045-363-1030. Fax: 045-363-1037.

TABLE 2. Colony color of isolates on each test medium employed

Bacteria	No. of strains tested	No. of strains in CT-SMAC medium that were colorless ^a	No. of strains in CT-SSMAC medium that were:		
			Red to pink	β -Galactosidase negative and colorless	β -Galactosidase positive and colorless ^a
<i>Escherichia coli</i> O157:H7 and O157:NM	19	19 (100) ^b	0 (0) ⁻	0 (0)	19 (100)
<i>Hafnia alvei</i>	2	2 (100)	0 (0)	0 (0)	2 (100)
Oxidase-negative strains	25	25 (100)	21 (84)	2 (8)	2 (8)
<i>Pseudomonas aeruginosa</i>	10	10 (100)	0 (0)	10 (100)	0 (0)
Oxidase-positive strains	64	64 (100)	0 (0)	59 (92)	5 (8)
All strains other than <i>Escherichia coli</i>	101	101 (100)	21 (21)	71 (70)	9 (9)

^a Including light pink strains.

^b The values in parentheses are percentages.

and the preparation was boiled and autoclaved at 121°C for 15 min. After sterilization and cooling to 50 to 55°C, 5 ml of a solution of Cefixime Tellurite Selectavial (Mast Group Ltd., Merseyside, United Kingdom) was added, and the preparation was mixed well and distributed in 20-ml portions into petri dishes (CT-SSMAC medium). CT-SMAC medium was also used.

All strains were incubated in buffered peptone water at 36°C for 18 h. After incubation, one loopful of each culture was spread separately onto CT-SMAC and CT-SSMAC media and incubated at 36°C for 20 to 22 h.

After incubation, the colors of colonies on the agar plates were determined. When acid was produced from salicin, a red to pink color developed. On the other hand, the fluorescence surrounding light pink to colorless colonies on CT-SSMAC medium was investigated by using a UV lamp. Light blue fluorescence indicated that a colony was β -galactosidase positive.

Table 2 shows growth and characteristics of colonies of the strains tested on the test media. Colonies of all of the *E. coli* O157:H7 and O157:NM strains on both of the media tested were colorless. Twenty-one (21%) of the strains that were not *E. coli* strains were red to pink on CT-SSMAC medium. Of 80 strains that produced colorless or light pink colonies on CT-SSMAC medium, 71 were β -galactosidase negative. Since these 71 strains were β -galactosidase negative, the 4-methylumbelliferyl- β -D-galactopyranoside was not hydrolyzed, and therefore, there were no chromogen molecules to fluoresce blue when they were irradiated with UV light. On the other hand, the colonies of all of the *E. coli* O157:H7 and O157:NM strains on CT-SSMAC were colorless and β -galactosidase positive. Ratnam et al. (12) reported that several carbohydrates, such as sorbitol, salicin, adonitol, inositol, and cellobiose, are not fermented by *E. coli* O157:H7 strains. Two of these carbohydrates, sorbitol and salicin, were used in our new selective medium. Chapman et al. (5) introduced sorbitol MacConkey medium containing cefixime and rhamnose for differentiation of *E. coli* O157 from sorbitol-negative *E. coli*. Compared to CT-SMAC, our new selective medium (CT-SSMAC medium) differentiated more strains from *E. coli* O157:H7 strains on the basis of colony color and β -galactosidase activity. Therefore, we recommend using CT-SSMAC medium to isolate *E. coli* O157:H7 from radish sprouts. Recently, an *E. coli* O157:H7 sorbitol-positive mutant has been described (7). Therefore, using other *E. coli* O157:H7 selective media along with CT-SSMAC medium is recommended.

E. coli is generally β -galactosidase positive, and the β -galactosidase reaction is helpful for identification of *E. coli* O157:H7. However, this reaction is not favorable when many colonies are grown on a single agar plate. The predominant gram-negative facultatively anaerobic rod-shaped bacteria (family *Enterobacteriaceae*) in normal human feces are *E. coli* strains (2–4, 6). Most strains of *E. coli* other than serotype O157:H7 strains produce acid from sorbitol (12). CT-SMAC medium is

useful for isolating *E. coli* O157:H7. On the other hand, many sorbitol-negative gram-negative facultatively anaerobic rods have been isolated from plants, including raw vegetables (8, 9). Therefore, using CT-SSMAC medium in investigations of the presence of *E. coli* O157:H7 in plants may be effective.

Aerobic bacteria, such as *Pseudomonas* spp., are also widely distributed in nature and in plants (10). The presence of these bacteria hinders isolation of *E. coli* O157:H7. Therefore, further studies may be necessary to establish other methods for inhibiting the growth of *Pseudomonas* spp. It would be very interesting to see if CT-SSMAC medium could be used for isolation of *E. coli* O157:H7 from raw vegetables other than radish sprouts.

This work was supported by Health Science Research grants from the Ministry of Health and Welfare in Japan.

REFERENCES

- Bennett, A. R., S. MacPhee, and R. P. Betts. 1995. Evaluation of methods for the isolation and detection of *Escherichia coli* O157 in minced beef. *Lett. Appl. Microbiol.* **20**:375–379.
- Benno, Y., K. Endo, T. Mizutani, Y. Namba, T. Komori, and T. Mitsuoka. 1989. Comparison of fecal microflora of elderly persons in rural and urban areas of Japan. *Appl. Environ. Microbiol.* **55**:1100–1105.
- Benno, Y., K. Sawada, and T. Mitsuoka. 1984. The intestinal microflora of infants: composition of fecal flora in breast-fed and bottle-fed infants. *Microbiol. Immunol.* **28**:975–986.
- Benno, Y., K. Suzuki, K. Suzuki, K. Narisawa, W. R. Bruce, and T. Mitsuoka. 1986. Comparison of the fecal microflora in rural Japanese and urban Canadians. *Microbiol. Immunol.* **30**:521–532.
- Chapman, P. A., C. A. Siddons, P. M. Zadik, and L. Jewes. 1991. An improved selective medium for the isolation of *Escherichia coli* O157. *J. Med. Microbiol.* **35**:107–110.
- Finogold, S. M., H. R. Attebery, and V. L. Sutter. 1974. Effect of diet on human fecal flora: comparison of Japanese and American diets. *Am. J. Clin. Nutr.* **27**:1456–1469.
- Fratamico, P. M., R. L. Buchanan, and P. H. Cooke. 1993. Virulence of an *Escherichia coli* O157:H7 sorbitol-positive mutant. *Appl. Environ. Microbiol.* **59**:4245–4252.
- Fujisawa, T., and M. Mori. 1992. Species of coliform bacteria isolated from market samples of raw vegetables. *J. Antibact. Antifung. Agents* **20**:251–253.
- Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams. 1994. *Bergey's manual of determinative bacteriology*, 9th ed., p. 175–289. The Williams & Wilkins Co., Baltimore, Md.
- Jay, J. M. 1978. The role and significance of microorganisms in nature and in foods, p. 9–27. *In* *Modern food microbiology*, 2nd ed. D. Van Nostrand Co., New York, N.Y.
- March, S. B., and S. Ratnam. 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J. Clin. Microbiol.* **23**:869–872.
- Ratnam, S., S. B. March, R. Ahmed, G. S. Bezanson, and S. Kasatiya. 1988. Characterization of *Escherichia coli* serotype O157:H7. *J. Clin. Microbiol.* **26**:2006–2012.
- Sata, S., R. Osawa, Y. Asai, and S. Yamai. 1999. Growth of starved *Escherichia coli* O157 cells in selective and non-selective media. *Microbiol. Immunol.* **43**:217–227.
- Zadik, P. M., P. A. Chapman, and C. A. Siddons. 1993. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *J. Med. Microbiol.* **39**:155–158.