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Use of 5-Bromo-4-Chloro-3-Indoxyl-ß-D-Glucuronide in MacConkey Sorbitol Agar to Aid in the Isolation of *Escherichia coli* 0157:H7 from Ground Beef

ANITA J. G. OKREND*, BONNIE E. ROSE, and CHARLES P. LATTUADA

Food Safety and Inspection Service, U.S. Department of Agriculture, Building 322, ARC-East, Beltsville, Maryland 20705

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ABSTRACT

The addition of 5-bromo-4-chloro-3-indoxyl-ß-D-glucuronide (BCIG) at the 0.1 g/L level, to MacConkey sorbitol agar (MSA) plates aided in the isolation of *Escherichia coli* 0157:H7 from raw ground beef samples by differentiating ß-glucuronidase positive from ß-glucuronidase negative colonies. *E. coli* 0157:H7 colonies, being sorbitol negative, ß-glucuronidase negative, remained white, while sorbitol negative, ß-glucuronidase positive colonies turned green to blue. Addition of BCIG to the MSA agar reduced the number of false suspect colonies picked from the primary plating medium by 36% when compared to MSA. *E. coli* 0157:H7 was isolated from 11 out of 12 inoculated meat samples (0.7 *E. coli* 0157:H7/g) using MSA-BCIG as compared to 8 out of 12 samples using MSA without BCIG.

Escherichia coli 0157:H7 has been recognized as an enteric pathogen and has been implicated in foodborne outbreaks of hemorrhagic colitis associated with the consumption of beef (*12,13,15*). For this reason, the isolation of this organism is important to both clinical and food microbiologists. A unique characteristic of this organism is its inability to ferment sorbitol (*8,14,18*), though 80.3% of *E. coli* strains are sorbitol fermenters (*2*). This characteristic has been utilized by both clinicians and food microbiologists to differentiate this serotype from other *E. coli* (*3,5,7,8,10,18*). We developed a method for isolation and identification of *E. coli* 0157:H7 from raw ground beef using MacConkey sorbitol agar (MSA) incubated at 42°C as the primary plating medium (*11*). Using this method in a screening program, we found that 25% of all sorbitol negative colonies picked, many of which were *E. coli*, were ß-glucuronidase positive. Since approximately 97% of all *E. coli* are ß-glucuronidase positive (*6*), but 0157:H7 is ß-glucuronidase negative (*8,14*), we decided that a detection system for this enzyme in the primary plating medium would be of great value. We rejected the use of 4-methylumbelliferyl-ß-D-glucuronide (MUG) for this purpose though it is the method commonly used for ß-glucuronidase detection, since the fluorescent product diffuses out of the colony, obscuring the actual colonial source of the enzyme.

Recent reports in the literature describe the use of the compounds 5-bromo-4-chloro-3-indoxyl-ß-D-glucuronide (BCIG) (*4,17*) and indoxyl-ß-D-glucuronide (IBDG) (*1,9*) to detect the production of ß-glucuronidase in bacterial colonies. Positive colonies grown on media containing these compounds turn blue with no diffusion of the color into the agar. Delisle and Ley (*1*) noted that in using IBDG to detect *E. coli*, they missed serotype 0157:H7 because these colonies did not develop the blue color. Similarly, Watkins et al. (*17*) reported that they missed a BCIG and MUG negative strain of *E. coli* due to lack of color development. We, therefore, decided to search for *E. coli* 0157:H7 by isolating white rather than blue colonies from MSA plates to which BCIG had been added.

MATERIALS AND METHODS

Media

MacConkey sorbitol agar (Difco 0079-17-7) was prepared according to manufacturer's instruction. To make MSA-BCIG, 0.1 g of 5-bromo-4-chloro-3-indoxyl-ß-D-glucuronic acid cyclohexylammonium salt (BCIG; Biosynth International, Skokie, IL) was dissolved in 2.5 ml of 95% ethanol - 0.5 ml of 1N NaOH and added to 1 liter of MSA (*17*). The complete medium was autoclaved at 121°C for 15 min, tempered, and poured into 150x15 mm petri dishes.

Modified EC broth with novobiocin (mEC+n) was prepared using the same formulation as EC broth, but the bile salts concentration was reduced to 1.12 g/L and a filter sterilized aqueous solution of sodium novobiocin (potency 890 µg/mg; Sigma N1628) was added to the sterile medium to produce a final concentration of 20 mg/L (*11*).

Phenol red sorbitol agar + MUG (PRS-MUG) was prepared by adding 0.5% D-sorbitol, 0.005% MUG (Biosynth International), and 2% agar to phenol red broth base (Difco, Detroit, MI). After autoclaving, deep plates (40 ml/petri dish) were poured (*11*).

Levine's eosin methylene blue agar plates (EMB; BBL, Cockeysville, MD) were prepared with extra agar for a final agar concentration of 3%. The additional agar prevented the swarming of *Proteus* over the agar surface (*11*).

Aerobic plate counts (35°C, APC) were done using the pour plate technique with plate count agar (PCA; Difco).

Preparation of meat samples

The meat samples used consisted of 25 g of retail ground beef formed into balls and inoculated with 0.6 to 0.7 *E. coli* 0157:H7/g (labeled B). Uninoculated 25-g samples were pre-

pared as controls (labeled A). All meat samples were held frozen 3 to 7 d before analysis (*11*).

Isolation method

Individual 25-g meat samples were placed into 225 ml of mEC+n broth in a Stomacher 3500 bag (Tekmar). The samples were stomachered 2 min in a Colworth Stomacher Blender 3500 (Tekmar) and incubated for 24 h at 35°C. After incubation, the cultures were serially diluted to 10^{-6} in Butterfield's Phosphate Diluent (BPD, *16*) and 0.1 ml from the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were spread plated on both MSA and MSA-BCIG plates (150x15 mm). The plates were incubated at 42°C overnight. A set consisting of one PRS-MUG and one EMB plate was identically labeled and gridded into 12 numbered sections for each set of MSA or MSA-BCIG plates. Sorbitol negative colonies (white) from the MSA plates, and sorbitol negative, BCIG negative colonies (white) from the MSA-BCIG plates were picked. Each colony selected was spread in the center of a section on the EMB plate and stabbed in the corresponding section of the PRS-MUG plate. The plates were incubated overnight at 35°C and were then examined for sorbitol fermentation, MUG reaction (fluorescence), and typical *E. coli* growth on EMB. Those cultures which were sorbitol negative, MUG negative, and typically *E. coli* were tested from the PRS-MUG plate with the Oxoid *E. coli* 0157 Latex Test. The 0157 positive cultures were then tested biochemically, and those that gave typical *E. coli* 0157:H7 reactions were then motility enhanced and tested serologically by a slide agglutination test for the H7 antigen (*11*).

RESULTS AND DISCUSSION

Initially 17 cultures, 5 meat isolates and 12 clinical isolates, of *E. coli* 0157:H7 were streaked on MSA-BCIG plates, incubated at 42°C, and the color reactions observed. All 17 cultures produced white colonies. Eight *E. coli* cultures that were sorbitol and ß-glucuronidase (MUG test) positive were tested in the same manner. These cultures produced growth that was a mixture of purple, red, and green colonies. In a third trial, six sorbitol negative and MUG positive meat isolates (genus unknown) produced colonies in various shades of green or blue. Only one culture which previously tested as MUG positive did not produce the blue-green color on MSA-BCIG. These reactions indicated that we were unlikely to get false negative reactions (color produced in an 0157:H7 colony).

To determine whether the BCIG might have an inhibitory effect on *E. coli* 0157:H7, comparative plate counts of two meat isolates and one clinical isolate were done on MSA, MSA-BCIG, and PCA. Duplicate sets of plates were incubated at 35 and 42°C. The results showed no inhibitory effect from the BCIG on the growth of these cultures at either temperature (Table 1).

The next step was to determine whether there would be an isolation advantage to using MSA-BCIG rather than MSA as the primary plating medium. Twelve packages of ground beef were obtained, each from a different retail establishment. Two 25-g samples were prepared from each package. One sample was inoculated with *E. coli* 0157:H7 at the 0.7/g level, the other served as the uninoculated control. The samples were frozen and held for 3 to 7 d, then tested for ease of isolation (as previously described). *E. coli* 0157:H7 was isolated from 8 of the 12 inoculated samples plated on MSA and 11 of the 12 inoculated samples plated on MSA-BCIG. *E. coli* 0157:H7 was not isolated from any of the control samples (Table 2).

TABLE 1. *Comparative counts per ml of three* E. coli *0157:H7 isolates plated on MSA, MSA-BCIG, and PCA incubated at 35°C and 42°C.*

E. coli 0157:H7	Numbers x 10^6					
	MSA		MSA-BCIG		PCA	
isolate	35°C	42°C	35°C	42°C	35°C	42°C
Meat	130	130	130	130	150	160
Meat	110	120	110	140	45	50
Clinical	68	82	67	74	120	130

From the analysis of the 24 ground beef samples plated on MSA, a total of 221 sorbitol negative colonies were picked, 91 of which proved to be MUG positive. Of the remaining 130 picks, 34 were confirmed as *E. coli* 0157:H7. From the analysis of the same ground beef samples plated on MSA-BCIG, a total of 142 sorbitol negative, BCIG negative colonies were picked. Only 4 of these colonies were MUG positive and of the remaining 139 colonies, 41 proved to be 0157:H7. In summary, from the total experiment, 15% of the picks from MSA and 29% of the picks from the MSA-BCIG were positive for 0157:H7.

In evaluating only the inoculated samples (B samples), 115 sorbitol negative colonies were picked from the MSA plates of which 51 were MUG positive and 34 were 0157:H7. The effective recovery rate of *E. coli* 0157:H7 from MSA

TABLE 2. *APC/g and numbers and characteristics of sorbitol negative colonies from MSA and sorbitol negative, BCIG negative colonies from MSA-BCIG for control (A) and inoculated (B) ground beef samples.*

Sample	APC (Log_{10})/g	MSA			MSA-BCIG		
		Total	MUG+	0157+	Total	MUG+	0157+
1A	4.84	9	8	0	4	0	0
1B		9	3	3	5	2	1
2A	5.34	0	0	0	0	0	0
2B		4	4	0	10	0	1
3A	2.81	2	1	0	0	0	0
3B		12	9	3	7	0	3
4A	5.32	10	2	0	1	0	0
4B		12	6	2	5	1	1
5A	6.20	12	12	0	1	0	0
5B		12	12	0	6	1	4
6A	6.18	12	12	0	6	0	0
6B		11	9	2	6	0	4
7A	5.98	2	2	0	0	0	0
7B		12	3	7	8	0	5
8A	6.11	4	0	0	1	0	0
8B		12	0	11	12	0	11
9A	5.49	12	2	0	3	0	0
9B		8	2	4	6	0	5
10A	6.28	12	1	0	12	0	0
10B		12	3	0	7	0	0
11A	5.43	9	0	0	8	0	0
11B		9	0	2	10	0	5
12A	4.26	12	0	0	12	0	0
12B		12	0	0	12	0	1
Totals		221	91	34	142	4	41

was 30%. From the inoculated samples spread on MSA-BCIG plates, 94 sorbitol negative, BCIG negative colonies were picked of which 4 were MUG positive and 41 were 0157:H7. The effective recovery rate of *E. coli* 0157:H7 from MSA-BCIG was 44%. From the control samples (A samples), 96 sorbitol negative colonies were picked from the MSA plates, 40 of which were MUG positive. None of the 48 sorbitol negative, BCIG negative colonies picked from the MSA-BCIG control sample plates were MUG positive.

The results of these experiments show that the addition of BCIG to MSA agar is very helpful in the isolation of *E. coli* 0157:H7. The compound does not differentiate those ß-glucuronidase negative, sorbitol negative colonies which are not *E. coli* from those that are *E. coli*. However, its presence did reduce by 36% the number of false suspect colonies picked from the primary plating medium and in so doing increased the chance of picking the appropriate colonies. The use of MSA-BCIG agar as the primary plating medium can greatly reduce the work necessary for testing large numbers of meat samples while increasing the efficiency of the isolation method.

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