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Short Communication

Evaluation of a chromogenic medium for total coliforms and *Escherichia coli* determination in ready-to-eat foods

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Abstract

Chromocult® coliform agar (CCA) was compared with the standard medium violet red bile agar (VRBA) for total coliform counts (TC) in different ready-to-eat foods. In addition, the specificity of CCA to detect *E. coli* was evaluated. CCA is a medium designed to detect TCs and *E. coli* by the specific action of β-D-galactosidase and β-D-glucuronidase. A total of 94 ready-to-eat food samples were obtained from restaurants, fast food shops and bars in Córdoba city, Argentina. The foods included boiled vegetable salads, cooked vegetables, meat and chicken meals, salad and sandwiches. One hundred and one strains were isolated at random from food samples. In CCA, the value of confirmation for TC was 81.3% and for *E. coli* was 93.3%. All *E. coli* strains isolated were β-D-glucuronidase positive. The median TC count was 4.61 log cfu/g on CCA and 3.58 log cfu/g on VRBA. The Spearman's correlation coefficient between TC count on VRBA and CCA was 0.72 ($P = 0.00001$). The Wilcoxon signed rank test revealed statistically significant differences between both variables. Disagreement between the two methods for TC enumeration was due to false positive results in CCA. The results of this study showed that CCA is an efficient method for simultaneous detection of *E. coli* and coliforms from ready-to-eat foods.

1. Introduction

E. coli is a bacterium whose natural habitat is the enteric tract of humans and warm-blooded animals (Kaspar et al., 1987). Thus, the presence of *E. coli* in foods is an indicator of direct or indirect fecal contamination. It is also an indicator of the possible presence of enteric pathogens in water, shellfish, dairy products and other foods. High counts of *E. coli* and total coliform (TC) in foods usually indicates lack of hygiene in handling and production operations, inadequate storage and post-process contamination (Blood and Curtis, 1995; de Sousa et al., 2002). Therefore, *E. coli* and TC enumeration are used as a food-quality parameter.

Solid medium method violet red bile agar (VRBA) and most probable number are standardized methods for coliform detection (APHA, 2001). Coliform tests are common practice; when these tests indicate possible fecal contamination, additional studies are performed to determine the presence of *E. coli* (ICMSF, 1982).

Diverse methods using chromogenic and/or fluorogenic substrates to reveal β-D-glucuronidase and β-D-galactosidase activity on culture media have been reported to determine whether a strain belongs to the coliform group and/or *E. coli*. Various commercial media containing chromogenic substrates for the identification and enumeration in 24 h of *E. coli* and TC are available: Chromagar® (Alonso et al., 1999) and Chromocult® (Geissler et al., 2000).

The VRBA culture medium allows coliform detection and enumeration in 24 h; however, it does not allow discrimination of *E. coli* from the rest of the coliforms (APHA, 2001). Chromocult® coliform agar (CCA) (Merck, Darmstadt, Germany) includes Tergitol®, which inhibits the growth of Gram-positive flora. It includes a mixture of chromogens: Salmon–Gal, which is hydrolysed by β-D-galactosidase to produce a reddish colour in coliform colonies, and X-gluc, hydrolysed by β-D-glucuronidase, giving a blue colour to the colonies. Both enzymes are synthesized by *E. coli,* so its colonies exhibit a dark blue to violet colour. False positive for

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TC were reported by Geissler et al. (2000) in a culture medium with chromogens. Alonso et al. (1999) found differences between chromogenic and standard medium for *E. coli* enumeration in river and marine waters.

This study was undertaken to compare CCA with standard media (VRBA) for TC counts in ready-to-eat foods. Also, the specificity of CCA was evaluated to detect *E. coli*.

2. Materials and methods

2.1. Food samples

A total of 94 ready-to-eat food samples were obtained from restaurants, fast food shops and bars in the city of Córdoba, Argentina. The foods included boiled vegetable salad, cooked vegetables, meat and chicken meals, salad and sandwiches. Approximately 100 g of each food was collected using sterile utensils and placed into sterile Whirl-Pak Bags (Nasco). The samples were immediately transported to the laboratory in an icebox (2–6°C). Bacteriological analysis was initiated 1–3 h after sampling. A 10 g analytical unit of each food was homogenized with 90 ml of sterile water-peptone (0.1%) for 2 min and then serial 10 fold dilutions were prepared with sterile water-peptone (APHA, 2001).

2.2. Total coliform enumeration on VRBA and CCA.

Duplicate 1 ml pour plates with a VRBA overlay were prepared using dilutions of each analytical unit. Plates were incubated at 35–37°C for 24–48 h. Dark red colonies with a red halo larger than 0.5 mm in diameter were considered to be coliform bacteria (APHA, 2001).

A 0.2 ml aliquot of the dilutions was cultured in CCA (Merck, Darmstadt) plates by the spreader method. Plates were incubated at 37°C for 24 h. Red to pink colonies were considered TC and dark-blue to violet colonies, presumptive *E. coli*. If plates from all dilutions had no colonies, the estimated count was reported as less than one times the corresponding lowest dilution.

Since Turner et al. (2000) and Geissler et al. (2000) reported that the heterotrophic bacteria interfere with detection of glucuronidase or β-D-galactosidase activity in *E. coli* and TC, cefsulodin solution was added to the medium, in order to reduce the level of accompanying flora.

2.3. Statistical treatment

TC counts obtained were transformed to log cfu/g. Results were analysed by correlation of paired data (Spearman's rank correlation). Analysis of variance was performed with Wilcoxon signed rank test. All statistical methods were performed with SigmaStat 2.03 software (SPSS Inc.).

2.4. VRBA and CCA culture media specificity

One hundred and one strains were isolated at random from the food samples. Seventy-two CCA and 29 VRBA colonies were isolated. The following assays were performed to determine whether these strains belonged to the *Enterobacteriaceae* family: oxidation fermentation test, cytochrome oxidase test, and Gram staining (Collins et al., 1991). All *Enterobacteriaceae* strains were identified with the API 20 E system (BioMérieux s.a. Marcy-L'Etoile, France). *E. coli* LAM1 control strain was used. The strains were simultaneously grown on CCA and VRBA plates by plating and stabbing methods. Plates were incubated at 37°C for 24 h.

3. Results and discussion

TC counts of 50% of the samples tested varied between 2.95 and 4.42 log cfu/g on VRBA and 3.54 and 5.03 log cfu/g on CCA. The total count range was 1.00–5.60 log cfu/g on VRBA and 2.18–6.21 log cfu/g on CCA. The median TC count was higher for the CCA (4.61 log cfu/g) than the VRBA (3.58 log cfu/g). Wilcoxon signed rank test revealed statistically significant differences between both variables. The Spearman's correlation coefficient between TC count on VRBA and TC count on CCA showed a positive correlation on a wide cfu/g range ($r = 0.72$; $P = 0.00001$). Disagreement between the two methods for TC enumeration was primarily due to false positive results on CCA.

The identification of isolated strains from CCA and VRBA is shown in Tables 1 and 2, respectively. The TC colonies from CCA were confirmed for lactose fermentation using VRBA. Of the 52 colonies β-galactosidase positive, 40 (77%) fermented the lactose. Of the isolates in VRBA, three *Pantoea* spp. strains were atypical (lactose negative) and β-D-galactosidase positive on CCA. Considering the strains isolated from VRBA and CCA that were β-D-galactosidase positive, there were 18.7% false positive (non-coliforms).

Some authors reported high a false positive TC rate using different media in water samples due to β-D-galactosidase positive colonies of *Aeromonas* spp., *Vibrio* spp. and *Serratia* spp.: 40.3% (Geissler et al., 2000), 25% (Covert et al., 1989) and 76% (Ley et al., 1993).

In this study, the value of confirmation for TC by CCA was 81.3%. False positive strains were *Serratia marcescens*, *Pantoea* spp., *Moellerella wisconsensis*, *Enterobacter gergoviae* and non-*Enterobacteriaceae* (18.7%). Since *S. marcescens* and *Pantoea* spp. do not ferment lactose, they would be excluded from the TCs;

Table 1
Identification of colonies isolated from CCA

Species	Colonies (%) from CCA				Colonies in VRBA[c]
	Red to pink[a]	Dark-blue to violet[a]	Light blue to turquoise[b]	White to yellow[b]	
Enterobacteriaceae					
Citrobacter freundii	1 (4)	—	—	—	T
Citrobacter koseri/farmeri	1 (4)	—	—	—	T
Enterobacter aerogenes	1 (4)	—	—	—	T
Enterobacter asburiae	1 (4)	—	—	—	T
Enterobacter gergoviae	3 (12)	—	—	—	A
Enterobacter sakazakii	2 (8)	—	—	—	T
Escherichia coli biotype 1	—	25 (93)	—	—	T
Hafnia alvei biotype 2	—	—	—	1 (8)	A
Klebsiella oxytoca	2 (8)	—	—	—	T
Kl. pneumoniae pnenumoniae	4 (16)	—	—	—	T
Klebsiella terrigena	1 (4)	—	—	—	T
Kluyvera sp.	—	—	—	1 (8)	A
Moellerella wisconsensis	1 (4)	—	—	—	A
Pantoea sp. biotype 2	1 (4)	—	—	—	A
Salmonella gallinarum	—	—	—	1[c] (8)	A
Serratia ficaria	—	—	1[c] (13)	—	A
Serratia marcescens	1 (4)	—	—	—	A
Serratia odorifera	1 (4)	—	—	—	T
Serratia plymuthica	1 (4)	—	—	—	T
Non-*Enterobacteriaceae*	4 (16)	2 (7)	7 (87)	9 (75)	N.D.
Total	25	27	8	12	

N.D.: Non determined.
[a] Typical colonies according to manufacturer.
[b] Atypical colonies according to manufacturer.
[c] Typical (T) or atypical (A) according to APHA (2001).

Table 2
Identification of colonies isolated from VRBA

Species	Colonies (%) from VRBA[a]		Colonies in CCA[b]
	Typical	Atypical	
Enterobacteriaceae			
Enterobacter cloacae	4(16)	—	Red
Enterobacter sakazakii	2(8)	—	Red
E. coli biotype 1	3(12)	—	Violet
Klebsiella oxytoca	2(8)	—	Red
Kl. pneumoniae pneumoniae	4(16)	—	Red
Klebsiella terrigena	7(28)	—	Red
Leclercia adecarboxilata	2(8)	—	Red
Pantoea sp. biotype 3	—	3(75)	Red
Serratia fonticola	1(4)	—	Red
Non-*Enterobacteriaceae*			
Myroides sp.	—	1(25)	Not grown
Total	25	4	

[a] Typical or atypical, according to APHA (2001).
[b] Red or violet colonies are typical according to manufacturer.

nevertheless, their capacity to hydrolyse galactoside leads to their erroneous inclusion within this group. Almost all of the strains of *M. wisconsensis* and *E. gergoviae* can ferment lactose (Holt et al., 1994); however, they exhibited atypical behaviour in VRBA. Only one of six false positive TC would be *Aeromonas* spp. (Gram-negative, oxidase positive). Turner et al. (2000) found a higher confirmation value for TC (93.7%) in a variety of meat products detected by the same method. *Kluyvera* spp. is an enteric species; more than 90% of the isolates can ferment lactose and are β-D-galactosidase positive (Holt et al., 1994). It would be expected that *Kluyvera* spp. grow as typical colonies in both media instead of developing white colonies in CCA and atypical in VRBA as observed (Table 1). *Salmonella gallinarum*, *Hafnia alvei* and *S. ficaria* are enteric species that do not ferment lactose (Holt et al., 1994). Therefore, they were expected to grow as atypical TC colonies, in accordance with the results of this study.

Twenty-seven strains isolates from CCA and three from VRBA demonstrated β-D-glucuronidase and β-D-galactosidase activity. Two strains were non-*Enterobacteriaceae*. Therefore, the confirmation rate of target blue to violet colonies as *E. coli* was 93.3%. Both β-D-glucuronidase and β-D-galactosidase activity were jointly found in two non-*Enterobacteriaceae* strains (6.7%). All of the *E. coli* isolated were β-D-glucuronidase positive. Manafi et al. (1991), in a review of chromogenic substrates, reported 94 to 96% of *E. coli*

isolates with β-D-glucuronidase positive reaction. A higher value (99%) was observed by Watkins et al. (1988) with a chromogenic compound. Venkateswaran et al. (1996) reported that of the 200 *E. coli* strains isolates, only two were found to be β-D-glucuronidase negative.

Alonso et al. (1999) found 91.3% and 92% confirmation rate for *E. coli* in river and marine samples water, respectively. They reported β-D-glucuronidase activity in some strains of *Citrobacter freundii*, *E. agglomerans* and *Klebsiella pneumoniae* using chromogenic medium incubated at 41°C. Turner et al. (2000) found a high confirmation rate (98.1%) for *E. coli* by CCA method in meat samples.

An atypical isolate was identified as *Myroides* spp. (non-*Enterobacteriaceae*). *Pantoea* spp. biotype 3 is lactose negative (Holt et al., 1994), a reason why they grew as atypical TC colonies in VRBA.

CCA and VRBA use different diagnostic principles for the detection of TC. CCA is principally based on the simultaneous detection of β-D-glucuronidase hydrolysis and β-D-galactosidase activity, whilst VRBA detects lactose fermentation. Thus, although both diagnostic and selective features used by CCA or VRBA are generally shared by coliforms, methodological differences are likely to influence the results.

In conclusion, CCA using chromogenic substrate to detect coliforms and *E. coli* has several advantages: only one medium is required to detect both TC and *E. coli*, reduced cost, significantly less processing time and less prone to cross-contamination than other standard methods. The results of this study showed that CCA is efficient for simultaneous detection of *E. coli* and coliforms from ready-to-eat foods.

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