Comparison of MI, Chromocult® coliform, and Compass CC chromogenic culture-based methods to detect *Escherichia coli* and total coliforms in water using 16S rRNA sequencing for colony identification

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ABSTRACT

The MI, Chromocult® coliform, and Compass CC chromogenic culture-based methods used to assess water quality by the detection of *Escherichia coli* and total coliforms were compared in terms of their specificity and sensitivity, using 16S rRNA sequencing for colony identification. A sewage water sample was divided in 2-μL subsamples for testing by all three culture-based methods. All growing colonies were harvested and subjected to 16S rRNA sequencing. Test results showed that all *E. coli* colonies were correctly identified by all three methods, for a specificity and a sensitivity of 100%. However, for the total coliform detection, the MI agar, Chromocult® coliform agar, and Compass CC agar were specific for only 69.2% (9/13), 47.2% (25/53), and 40.5% (17/42), whereas sensitive for 97.8% (45/46), 97.5% (39/40), and 85.7% (24/28), respectively. Thus, given the low level of specificity of these methods for the detection of total coliforms, confirming the identity of total coliform colonies could help to take public health decisions, in particular for cities connected to a public drinking water distribution system since the growth of few putative total coliform colonies on chromogenic agar is problematic and can lead to unnecessary and costly boiling notices from public health authorities.

Key words | 16S rRNA sequencing, Chromocult® coliform agar, Compass CC agar, MI agar, water

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INTRODUCTION

To assess water quality, methods based on the enzymatic properties of coliforms are used. The β-galactosidase enzyme is used because conventional coliform monitoring is based on the detection of the presence of β-galactosidase whereas the β-glucuronidase enzyme is used because the gene encoding this enzyme (*uidA*) was found to be specific (Brenner *et al.* 1972) and present in more than 97% of *Escherichia coli* isolates (Lupo & Halpern 1970; Martins *et al.* 1993). These chromogenic media were mostly developed to circumvent the need for a confirmation stage that

is required by both multiple-tube fermentation and membrane filter techniques and to diminish background effects of heterotrophic bacteria (Clark 1980; Evans *et al.* 1981; Means & Olson 1981; Seidler *et al.* 1981; Burlingame *et al.* 1984; APHA/AWWA/AEF 2005).

The MI agar (BD, Franklin Lakes, NJ, USA), Chromocult® coliform agar (Merck KGaA, Darmstadt, Germany), and Compass CC agar (Biokar Diagnostics, Allonne, Beauvais, France) are three commercial test methods based on the determination of β-galactosidase and β-glucuronidase

enzyme activities used to detect, within 24 h, *E. coli* and total coliforms in water samples. These tests are easy to use, require no additional confirmatory step and provide a more rapid estimate of indicators of the bacteriological contamination of water compared to classical techniques (Edberg *et al.* 1988; Brenner *et al.* 1993, 1996a, 1996b; Horman & Hanninen 2006; Pitkanen *et al.* 2007). Different collections of strains were tested with each commercial β-galactosidase and β-glucuronisade-based test method to establish their ability to recover total coliforms and *E. coli* strains. All of these methods were found to be at least as efficient as classical reference methods (Rice *et al.* 1990, 1991, 1993; Landre *et al.* 1998).

Before the use of chromogenic culture-based methods, the presence of high numbers of background heterotrophic bacteria and excessive crowding of colonies was shown to decrease coliform recovery by membrane filtration techniques using mEndo LES agar (Clark 1980; Burlingame *et al.* 1984). The most prevalent background heterotrophic bacteria on non-chromogenic culture-based methods have been identified as *Aeromonas hydrophila*, *Acinetobacter* sp., and *Pseudomonas aeruginosa* (Clark 1980; Burlingame *et al.* 1984). Since background bacteria could interfere with the growth and the detection of target microorganisms, Maheux *et al.* (2014b) tested the ability of MI agar, Chromocult® coliform agar, and DC with BCIG agar chromogenic membrane filtration culture-based methods to detect *E. coli* colonies on plates containing an increasing concentration of a non-target bacterium. They showed that the growth of *E. coli* colonies on these three chromogenic culture-based methods is inhibited by the growth of high concentrations of non-target microorganisms. They also showed that the *E. coli* detection was influenced by (1) the chromogenic agents chosen and (2) the composition of the medium. However, Maheux *et al.*'s (2014b) study was conducted using a total coliform strain and bacteria found as heterotrophic on non-chromogenic culture-based methods. Thus, even if the level of inhibition using non-target microorganisms has been established, the results obtained may not reflect totally the situations observed using real water samples since atypical bacteria growing on chromogenic culture-based methods could be different to those tested by Maheux *et al.* (2014b).

To our knowledge, no study has yet been conducted to identify the heterotrophic bacteria that grow on different chromogenic culture-based methods that could interfere with the growth and detection of *E. coli* and total coliforms. Furthermore, there is no study comparing the limits (specificity, sensitivity) of these three methods using 16S rRNA sequencing as the gold standard for colony identity.

In this study, we first compared MI agar, Chromocult® coliform agar, and Compass CC agar, in terms of specificity and sensitivity, using 16S rRNA sequencing to identify growing colonies. Second, we compared the three methods for their ability to limit the growth of atypical colonies, ease of use, and affordability. The identity of atypical colonies harvested for each method has also been investigated.

MATERIALS AND METHODS

Water sample

The sewage water sample used in this study was harvested at the discharge of the grit chambers of the west wastewater treatment plant of Québec City, in December 2014.

Membrane filtration methods

The membrane filtration method was performed according to Maheux *et al.* (2009). Three 2 µL volumes of sewage water were diluted in 25 mL of distilled water and filtered on mixed cellulose ester filters (47 mm diameter, 0.45 µm pore size; Millipore Corporation, Billerica, MN, USA) with a standard platform manifold. The first filter was incubated on MI agar (MI; BD, Franklin Lakes, NJ, USA), the second filter was incubated on Chromocult® coliform agar (Merck KGaA, Darmstadt, Germany), and the third filter was incubated on Compass CC agar (Biokar Diagnostics, Allonne, Beauvais, France) for 24 h ± 2 h at $35.0 \pm 0.5\,°C$, before determining colony counts and color. Furthermore, all colonies were recovered for genotypic identification. Each preparation of MI, Chromocult® coliform, and Compass CC plates was tested for performance using pure cultures of target and non-target microorganisms, as

recommended by the USEPA microbiology methods manual. Tests to confirm the sterility of the filter membranes and buffer used for rinsing the filtration apparatus were also performed (APHA/AWWA/AEF 2005).

Preparation of DNA extract for genotypic identification

DNA extracts of recovered colonies were realized using a bacterial suspension adjusted to a 0.5 McFarland standard (Fisher Scientific Company, Ottawa, Ontario, Canada). The cells were lysed using the BD Diagnostics-GeneOhm Rapid Lysis kit as recommended by the manufacturer (BD Diagnostics-GeneOhm, Québec, Québec, Canada).

Genotypic identification

The identity of the colonies isolated on MI, Chromocult® coliform, and Compass CC plates was confirmed by nucleotide sequencing of 16S rRNA gene using amplification and sequencing primers: SSU27 (5′-AGAGTTTGATCMTGGCT-CAG-3′) and SSU534R (5′-ATTACCGCGGCTGCTGG-3′). Oligonucleotide primers and probes were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

Briefly, 1 μL of the DNA extract was transferred directly to a 49 μL polymerase chain reaction (PCR) mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 mM each primer, 200 mM each deoxyribonucleoside triphosphate (GE Healthcare Bio-Sciences Inc., Baie d'Urfé, Québec, Canada), 3.3 mg per mL of bovine serum albumin (BSA; Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada), 0.06 μg/μL methoxsalen (Sigma-Aldrich Canada Ltd), 0.5 U of Taq DNA polymerase (Promega, Madison, WI, USA), and TaqStart antibody (Clontech Laboratories, Mountain View, CA, USA). Decontamination of the PCR mixtures prior to PCR was achieved using the UV crosslinker Spectrolinker model XL-1000 (Spectronics Corporation, Westbury, NY, USA; Isabel *et al.* 2008). For each experiment, 1 μL of sterile water was added to the PCR mixture as negative control. These PCR mixtures were subjected to thermal cycling (3 min at 95 °C and then 40 cycles of 1 s at 95 °C, 60 s at 58 °C, and 60 s at 72 °C) with a Mastercycler PRO (Eppendorf Canada Ltd, Mississauga, ON, Canada).

Sequencing of the 16S rRNA gene was performed as described by Picard *et al.* (2004). Molecular analysis of sequences was conducted using NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi, Standard Nucleotide BLAST optimized for highly similar sequence) and the Ribosomal database project (http://rdp.cme.msu.edu/, isolates with good quality sequence in Sequence Match search).

RESULTS AND DISCUSSION

Ability of MI agar, Chromocult® coliform agar, and Compass CC agar to detect *E. coli* and total coliforms from a sewage water sample

Three 2 μL of a 1-L sewage water sample harvested at the discharge of the grit chambers of the west wastewater treatment plant of Québec City were used for testing by MI agar, Chromocult® coliform agar, and Compass CC agar methods to verify their comparative ability to detect *E. coli* and total coliforms (Table 1). All these colonies were harvested and subjected to molecular identification using a 527 base-pairs fragment of the 16S rRNA gene. For the three culture-based methods tested, all blue colonies were identified as *E. coli*. Furthermore, none of the non-blue colonies was identified as *E. coli*, for a specificity and a sensitivity of 100%. However, MI agar and Compass CC agar detected, respectively, 12 and 13 *E. coli* colonies, whereas Chromocult® coliform agar detected 7 *E. coli* colonies. This result is in accordance with Maheux *et al.* (2015), demonstrating that for the detection of *E. coli*-positive water samples, all enzymatic culture-based methods tested were statistically equivalent.

For total coliform detection, 49 fluorescent (phenotype +) and 10 non-fluorescent (phenotype −) colonies were harvested for a total of 59 colonies on MI agar. On Chromocult® coliform agar, 67 dark blue/purple/red (phenotype +) and 26 colorless (phenotype −) colonies were harvested for a total of 93 colonies. Finally, on Compass CC agar, 49 purple/pink (phenotype +) and 21 white (phenotype −) colonies were harvested for a total of 70 colonies. All these colonies were harvested and subjected to molecular identification using the 16S rRNA gene. Molecular analysis

Table 1 | Specificity, sensitivity, and predictive values of each method for the detection of total coliform as compared to 16S rRNA gene identification

	16S rRNA identification					
Method and results	**+**	**−**	**Specificity[a]**	**Sensitivity[b]**	**Positive predictive value[c]**	**Negative predictive value[d]**
MI agar						
+	45	4	69.2%	97.8%	91.8%	90.0%
−	1	9				
Chromocult® coliform agar						
+	39	28	47.2%	97.5%	58.2%	96.2%
−	1	25				
Compass CC agar						
+	24	25	40.5%	85.7%	49.0%	81.0%
−	4	17				

[a]No. of true negative results/(no. of true negative + false positive results).
[b]No. of true positive results/(no. of true positive + false negative results).
[c]No. of true positive results/(no. of true positive + false positive results).
[d]No. of true negative results/(no. of true negative + false negative results).

showed that four fluorescent (4/49) colonies on MI agar, 28 dark blue/purple/red (28/67) colonies on Chromocult® coliform agar, and 25 purple/pink (25/49) colonies on Compass CC agar were not total coliforms for a false positive rate of 8.2%, 41.8%, and 51.0%, respectively. On the contrary, there were one non-fluorescent (1/10) colony on MI agar, one colorless (1/26) colony on Chromocult® coliform agar, and four white (4/21) colonies on Compass CC agar for a false negative rate of 10.0%, 3.8%, and 19.0%, respectively. By using 16S rRNA molecular identification as the gold standard, MI agar method has presented the highest specificity (69.2%), sensitivity (97.8%), and positive predictive value (91.8%; Table 1) for the detection of total coliforms. The highest negative predictive value has been obtained by the Chromocult® coliform agar method. Finally, MI agar and Chromocult® coliform agar detected 45 and 39 total coliforms colonies, respectively, whereas Compass CC agar only detected 22 total coliform colonies. This observation is in accordance with Maheux *et al.* (2014a), in which the MI agar method presents the highest recovery rate of total coliforms among MI agar, Chromocult® coliform agar, Colilert®, and DC with BCIG agar in controlled laboratory experiments using pure cultures. A statistical analysis realized by Maheux *et al.* (2015) also showed that Colilert and DC with BCIG agar detected less total coliform colonies than MI agar method using well water samples.

Population of total coliforms per genera detected by MI agar, Chromocult® coliform agar, and Compass CC agar isolated from the same sewage water sample

After the colonies were identified using 16S rRNA analysis, the population of total coliforms detected by MI agar, Chromocult® coliform agar, and Compass CC agar were classified per genera. Results showed that the population detected was more extended for MI agar and Chromocult® coliform agar (nine and seven different genus, respectively) than Compass CC agar (only four different genus; Table 2). Our results also showed a larger population within the species detected by each method (data not shown). Like the Maheux *et al.* (2008) study, the results of the present study showed the lack of correlation between test methods based on the same enzymatic principle to recognize a strain as non-*E. coli* total coliform. Indeed, our results showed that there is a weak correlation between the three methods tested either within the same genera or the same species. Since all colonies of the present study were isolated from the same water sample and treated in the same way (filtration, incubation, etc.), the difference observed in the population of strains detected by each method cannot just be attributed to environmental factors. The composition of each medium is also involved. This result is in accordance with Maheux *et al.* (2014b), who

Table 2 │ Population of total coliform per genera detected by MI agar, Chromocult® coliform agar, and Compass CC agar from 2 μL of sewage water

MI agar			Chromocult® coliform agar			Compass CC agar		
Species	**No.**	**%**	**Species**	**No.**	**%**	**Species**	**No.**	**%**
Escherichia spp.	14	31.1	*Klebsiella* spp.	11	28.2	*Escherichia* spp.	14	63.6
Enterobacter spp.	10	22.2	*Enterobacter* spp.	10	25.6	*Klebsiella* spp.	5	22.7
Klebsiella spp.	6	13.3	*Escherichia* spp.	9	23.1	*Citrobacter* spp.	2	9.1
Yersinia spp.	5	11.1	*Citrobacter* spp.	5	12.8	*Enterobacter* sp.	1	4.5
Citrobacter spp.	4	8.9	*Yersinia* spp.	2	5.1			
Kluyvera spp.	3	6.7	*Kluyvera* sp.	1	2.6			
Rahnella sp.	1	2.2	*Raoultella* sp.	1	2.6			
Raoultella sp.	1	2.2						
Serratia sp.	1	2.2						
Total	45	100.0		39	100.0		22	100.0

showed that the *E. coli* detection is influenced by the composition of the medium.

Growth of atypical colonies

All atypical colonies, also called background heterotrophic bacteria, harvested on MI agar, Chromocult® coliform agar, and Compass CC agar, were identified using 16S rRNA sequencing. To our knowledge, this is the first report of the identity of background heterotrophic bacteria detected on chromogenic culture-based methods. The amount of atypical colonies detected on each culture-based method was different. While MI agar detected only 8 atypical colonies, Chromocult® coliform agar and Compass CC agar detected, respectively, 27 and 17 colonies (Table 3). Furthermore, while MI agar detected mostly a

bacterium called *Uruburuella suis* as the atypical colony, the population detected was larger for Chromocult® coliform agar and Compass CC agar where the most prevalent atypical colony was an *Aeromonas* spp. (85.2% and 47.1%, respectively). Thus, according to Maheux *et al.* (2015), the MI agar method is composed of the more stringent medium since concentrations of atypical colonies growing on MI agar are less important than the two other methods.

Time to result, ease of use, and affordability

In terms of time to result, all three methods tested comparably since they required 24 hours for results without a confirmation step.

The MI agar, Chromocult® coliform agar, and Compass CC agar methods also provided comparable ease of use in

Table 3 │ Population of background heterotrophic bacteria detected by MI agar, Chromocult® coliform agar, and Compass CC agar from 2 μL of sewage water

MI agar				Chromocult® coliform agar				Compass CC agar			
Species	**No.**	**%**	**%ᵃ**	**Species**	**No.**	**%**	**%ᵃ**	**Species**	**No.**	**%**	**%ᵃ**
Uruburuella spp.	7	87.5		*Aeromonas* spp.	23	85.2		*Aeromonas* spp.	8	47.1	
Morganella sp.	1	12.5		*Uruburuella* spp.	2	7.4		*Pseudomonas* spp.	4	23.5	
				Acinetobacter sp.	1	3.7		*Acinetobacter* spp.	2	11.8	
				Lelliottia sp.	1	3.7		*Shewanella* spp.	2	11.8	
								Uruburuella sp.	1	5.9	
Total	8	100.0	13.6		27	100.0	28.0		17	100.0	24.3

ᵃPercentage of background heterotrophic bacteria on each plate.

terms of membrane filtration methods. Medium must also be prepared and quality control carried out for each batch. Employees already using membrane filtration equipment can easily use these methods.

In terms of affordability, all three methods are comparable. Indeed, the cost of medium and the cost associated with employees is more or less the same.

CONCLUSION

In this study, we compared the MI agar, Chromocult ®coliform agar, and Compass CC agar methods in terms of specificity, sensitivity, and their ability to limit the growth of atypical colonies using a sewage water sample. To our knowledge, this is also the first report regarding identification of background heterotrophic bacteria growing on these media. We showed that the use of the MI agar method seems to be the best option for the assessment of water quality by total coliform and *E. coli* detection. Indeed, globally, MI agar is more specific, more sensitive, and more able to limit the growth of atypical colonies than Chromocult® coliform and Compass CC agar.

Furthermore, given the low level of specificity of these methods for the detection of total coliforms, confirming the identity of total coliform colonies could help in taking public health decisions, in particular for cities connected to a public drinking water distribution system since the growth of few putative total coliform colonies on chromogenic agar is problematic and can lead to unnecessary and costly boiling notices from public health authorities.

The results obtained in the present study were obtained using sewage water samples. Results could differ with other types of water.

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