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Tellurite resistance profiles and performance of different chromogenic agars for detection of non-O157 Shiga toxin-producing *Escherichia coli*

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) are globally important food-borne pathogens. The isolation of non-O157 STEC is a significant public health challenge due to the dramatic diversity of their phenotypes and genotypes. In the present study, 476 non-O157 STEC strains representing 95 different O-serogroups were used to evaluate tellurite resistance and the performance of 12 different chromogenic agars. Of 476 strains, only 108 (22.7%) strains showed the minimal inhibitory concentration (MIC) values for potassium tellurite being higher than 4 μg/ml, and 96 (20.2%) strains harbored intact *ter* genes cluster. The presence of *ter* genes was significantly correlated with tellurite resistance. Six commercial chromogenic agars (TBX, MAC, SMAC, Rainbow® Agar O157, CHROMagar™ ECC, and Fluorocult O157) supported the growth of all strains. However, CT-SMAC, CHROMagar™ O157, and CHROMagar™ STEC agars exhibited 12.2%, 31.1%, and 38.0% of growth inhibition, respectively. Furthermore, 4.6%, 33.2%, and 45.0% of strains were inhibited on RBA-USDA, RBA-NT, and BCM O157 agar media. Variations in tellurite resistance and colony appearance might result in discrepant performance of non-O157 STEC recovery from different chromogenic agars. Using inclusive agars or less selective agar in combination with highly selective agar should be suggested to recover most non-O157 STEC strains, which would increase the probability of recovering STECs from complex background microflora.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are widely recognized as foodborne pathogens that produce either Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2) or both toxins, causing human gastrointestinal disease and outbreaks of diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS) worldwide (Grisaru et al., 2017). Since the first STEC O157: H7 outbreak was reported in the United States (Riley et al., 1983), sporadic infections or outbreaks caused by non-O157 STECs have increased dramatically in many countries recently (Buvens et al., 2012; Gould et al., 2013; Kanayama et al., 2015; Robertson et al., 2016). This is especially notable for *E. coli* O104:H4, which was responsible for the outbreak linked to fenugreek sprouts in Germany resulting in over 3000 cases of infection and 50 deaths (Beutin and Martin, 2012). Stx is regarded as the most critical virulence factor of STEC. Another virulence factor, intimin (*eae*), is located on the locus of enterocyte effacement (LEE) and also contributes to high pathogenicity (Smith et al., 2014). Ruminants, especially cattle, are the major reservoir for STEC (Huang, 2012). Humans typically get infected by ingestion of food or water contaminated with animal feces.

Being able to reliably detect STEC organisms could assist and improve surveillance activities, including the capture of emergent strains. For rapid detection of non-O157 STECs in food matrices, new techniques have been broadly explored (Kim et al., 2017; Park et al., 2014). STEC O157 exhibits some biochemical characteristics that are different from other *E. coli*. For example, most STEC O157 does not ferment sorbitol, so Sorbitol-MacConkey (SMAC) agar is widely used by various laboratories (Fedio et al., 2011; Yoshitomi et al., 2012). However, due to the dramatic biochemical diversity of non-O157 STECs and their inherent sensitivities to additives, the standardized method for reliable and effective isolation of all non-O157 STECs has not yet been proposed. A number of agar media designed to detect non-O157 STECs rely on fermentation of specific carbohydrates, specific enzyme activity, and/or resistance to additives (Kalchayanand et al., 2013). Tryptone Bile X-glucuronide agar (TBX) is recommended by the International Organization for Standardization (ISO/TS 13136:2012) for the

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detection of STEC serogroups O157, O111, O26, O103 and O145 (Harakudo et al., 2016). Two chromogenic agars that target *E. coli* O157:H7, i.e. Rainbow® Agar O157 and R&F® *E. coli* O157:H7 agar, are recommended in the FDA BAM for the recovery of STEC (www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm). The USDA FSIS researchers found that a modified Rainbow® Agar O157, containing substantially less tellurite and novobiocin, enabled better recovery than the standard version of Rainbow® Agar O157 (Tillman et al., 2012). In addition, the performance of CHROMagar™ STEC for the detection of non-STEC was assessed (Stromberg et al., 2016; Verhaegen et al., 2015; Wylie et al., 2013; Zelyas et al., 2016).

Successful detection of STEC in a complex background requires an agar medium that suppresses the growth of background microorganisms with minimal suppression of target STECs (Stromberg et al., 2016). For this purpose, various additives are selected, and potassium tellurite (K_2TeO_3) is a major ingredient (Kerangart et al., 2016). Tellurite resistance in STEC O157 is encoded by the *ter* gene cluster, which comprises eight genes, i.e. *terZ*, *terA, terB*, *terC, terD*, *terE*, *terF*, and *terW* (Taylor, 1999). The *ter* genes are commonly present in clinical STEC O157 isolates (Bielaszewska et al., 2005; Taylor, 1999). However, some clinically relevant non-O157 STEC such as O91 and O113 strains cannot grow on standard media containing 2.5 μg/ml of K_2TeO_3 (Orth et al., 2007). Evaluation of commercial media containing K_2TeO_3 also showed significant inhibition of some non-O157 STECs, such as O103, O111, O121 and O145 strains (Gill et al., 2014; Gould et al., 2013; Hirvonen et al., 2012; Tzschoppe et al., 2012). Therefore, the use of potassium tellurite as additives for isolation of non-O157 STECs should also be carefully considered.

Several studies have investigated the presence and expression of *ter* genes in non-O157 STEC, but only in a small number of serogroups related to clinically severe diseases such as O111, O26 and O103 (Kerangart et al., 2016; Orth et al., 2007). In this study, we investigated the presence of *ter* genes and tellurite resistance in a broad range of serotypes of non-O157 STEC strains isolated from various sources. We then evaluated the growth performance on 12 chromogenic media based on the fermentation of carbohydrates, β-glucuronidase activity, and/or resistance to addition of antimicrobials. Our work is dedicated to provide a suggestion of chromogenic media for the isolation of STEC of various serotypes, which is crucial in facilitating rapid detection of non-O157 STECs in outbreak investigations or as part of ordinary surveillance of these emerging pathogens.

2. Materials and methods

2.1. Bacterial strains

A total of 476 non-O157 STEC strains used in this study were obtained from our laboratory collection during April 2009 to August 2016 in China. Of these, 351, 60, 30 and four strains were isolated from animals, food, diarrheal patients, and healthy carriers, respectively. The remaining 31 strains were isolated from samples collected by local centers for disease control and prevention and the sources were unknown. All strains were isolated and identified using previously described methods (Bai et al., 2016) and stored at −80 °C. It is noteworthy that only a few isolates were recovered from CHROMagar™ STEC agar (CHROMagar, Paris, France), a tellurite-amended medium. Most strains were isolated from CHROMagar™ ECC and/or MacConkey agar (Land Bridge, Beijing, China), two inclusive agars.

2.2. Serotyping and detection of eae gene

The O:H serotype of each isolate was determined by methods as described previously (Bai et al., 2016). The intimin-encoding gene *eae* from all 476 non-O157 STEC isolates was screened according to a previously reported PCR-based method (Bai et al., 2013).

2.3. Recovery of STEC strains on different chromogenic agars

The following 12 commercial chromogenic agars were evaluated for their ability to support the growth of non-O157 STEC strains. TBX agar (Oxoid, Hampshire, UK); MacConkey agar (MAC, Land Bridge, Beijing, China); sorbitol MacConkey agar (SMAC, Oxoid, Hampshire, UK); CHROMagar™ ECC (CH-ECC, CHROMagar, Paris, France); Sorbitol MacConkey agar containing 0.05 μg/ml cefixime and 2.5 μg/ml potassium tellurite (CT-SMAC); Rainbow® Agar O157 (RBA, Biolog Inc., Hayward, California, USA); Rainbow® Agar O157 supplemented with 10 μg/ml novobiocin and 0.8 μg/ml potassium tellurite (RBA-NT); Rainbow® Agar O157 supplemented with 5 μg/ml novobiocin, 0.05 μg/ml cefixime trihydrate and 0.15 μg/ml potassium tellurite (RBA-USDA); CHROMagar™ STEC (CH-STEC) and CHROMagar™ O157 (CH-O157) with unavailable composition of supplement (CHROMagar, Paris, France); BCM O157:H7 supplemented with 0.8 μg/ml potassium tellurite and 10 μg/ml novobiocin (BCM, Biosynth AG, Staad, Switzerland); Fluorocult® *E. coli* O157:H7 (Fluorocult O157) without supplement (Merck, Darmstadt, Germany). Briefly, approximately a loopful of each culture grown in Luria-Bertani (LB) medium (Land Bridge, Beijing, China) was streaked onto the above agar media, and incubated for 18–24 h at 37 °C. The colony morphologies on these agars were recorded. Collectively, three growth patterns were observed and defined according to the presence of the colony in different inoculating zones: good growth (colonies appeared in all inoculating zone), inhibited growth (colonies only observed in the first inoculating zone) and no growth (no obvious colonies).The experiment was performed in triplicate for each isolate.

2.4. Tellurite susceptibility testing

Tellurite minimal inhibitory concentrations (MICs) were determined using a microdilution agar method (Taylor et al., 2002). Briefly, a single colony was streaked onto LB medium and incubated overnight at 37 °C with shaking. 30 μl culture was transferred into 3 ml fresh LB broth (1:100 dilution) and incubated at 37 °C to a concentration of 10^8 CFU/ml (ca. A_{600} = 0.5). Ten microliter phosphate-buffered saline diluted culture (ca. 10^5 CFU/ml) was spotted onto LB agar plates containing serial two-fold dilutions of K_2TeO_3 from 1 to 1024 μg/ml. Plates were incubated at 37 °C for 18–24 h under aerobic growth conditions. The MIC was determined as the lowest dilution of potassium tellurite that completely inhibited growth. Tellurite-resistant *E. coli* O157:H7 strain Sakai and tellurite-sensitive *E. coli* strain ATCC®25922 were used as controls. Each strain was tested in triplicate and in two independent experiments.

2.5. Testing of tellurite-resistant genes

All 476 non-O157 STEC isolates were subjected to screening for tellurite-resistant genes (*terA*, *terB*, *terC*, *terD*, *terE*, *terF*, *terW*, and *terZ*) by PCR as described previously (Taylor et al., 2002). For all PCRs, *E. coli* O157 strain Sakai was used as a positive control and *E. coli* ATCC® 25922 served as a negative control.

2.6. Statistical analysis

Statistical analysis was performed by using the χ^2 and Fisher's exact probability test. SAS® 9.3 (SAS Institute Inc., North Carolina, USA) was used to perform calculations. A P value < 0.05 was considered to be statistically significant.

3. Results

3.1. Bacterial growth on commercial selective media

After 24 h incubation, three growth patterns were observed on 12