Journal of Food Safety and Food Quality 66, Heft 2 (2015), Seiten 25–52

Arch Lebensmittelhyg 66,
28–32 (2015)
DOI 10.2376/0003-925X-66-28

ISSN 0003-925X

Korrespondenzadresse:
stephanr@fsafety.uzh.ch

55. DVG Meeting 2014 Special:

Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich,
Zurich, Switzerland

Evaluation of the CHROMagar™ STEC medium for the detection of Shiga toxin-producing *Escherichia coli*

Evaluierung des CHROMagar™ STEC Mediums für den Nachweis von Shigatoxin-bildenden Escherichia coli

Nicole Cernela, Claudio Zweifel, Roger Stephan

Summary

Fast and reliable isolation of Shiga toxin-producing *Escherichia coli* (STEC) remains a challenge in routine diagnostics. The present study evaluated the performance of the CHROMagar™ STEC medium by testing the growth capacity of 39 STEC strains from human patients and of 35 non-target strains. The majority (83.3 %) of the 18 STEC strains belonging to the top-five serogroups (O26, O103, O111, O145, O157) did grow on and showed typical mauve colonies (exceptions were one O26 isolate and two sorbitol-fermenting non-motile O157 isolates). However, only five of the 21 STEC strains not belonging to the top-five serogroups showed growth and typical colonies. Of the 28 *stx*-negative *E. coli* strains (including 13 O157 isolates) and the seven non-*E. coli* strains, 10 *stx*-negative/*eae*-positive *E. coli* strains of serogroups O2, O26, O113, O128, O145 and O177 did grow and showed typical colonies. Thus, the CHROMagar™ STEC medium can not be recommended as a primary STEC screening method in routine diagnostics. The CHROMagar™ STEC medium should therefore only be used for specific questions or in routine STEC diagnostics in combination with another method or medium.

Keywords: CHROMagar™ STEC, Shiga toxin-producing *E. coli,* serotypes, growth capacity, colony morphology

Zusammenfassung

Die schnelle und zuverlässige Isolierung von Shigatoxin-bildenden *Escherichia coli* (STEC) stellt in der Routinediagnostik eine grosse Herausforderung dar. Die vorliegende Arbeit untersuchte das Wachstum von 39 STEC-Stämmen und 35 non-STEC-Stämmen auf dem CHROMagar™ STEC Medium. Die Mehrheit (83.3%) der 18 STEC-Stämme, die zu den Top-Fünf STEC-Serogruppen (O26, O103, O111, O145, O157) gehörten, zeigte Wachstum typischer, violetter Kolonien (Ausnahmen waren ein O26 Stamm und die zwei Sorbitol-fermentierenden O157:H^- Stämme). Bei den übrigen 21 STEC-Stämmen ergab sich ein anderes Bild: Nur bei fünf lag Wachstum typischer Kolonien vor. Von den non-STEC-Stämmen (28 *stx*-negative *E. coli*-Stämme und sieben nicht-*E. coli*-Stämme) zeigten 10 *stx*-negative/*eae*-positive *E. coli*-Stämme der Serogruppen O2, O26, O113, O128, O145 und O177 ebenfalls Wachstum typischer Kolonien. Basierend auf diesen Ergebnissen kann das CHROMagar™ STEC Medium nicht als primäre STEC-Screening-Methode empfohlen werden. Dieses Medium sollte daher nur im Rahmen spezifischer Fragestellungen oder in der Routinediagnostik in Kombination mit einer anderen Methode eingesetzt werden.

Schlüsselwörter: CHROMagar™ STEC, Shigatoxin-bildende *E. coli,* Serotypen, Wachstum, Koloniemorphologie

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are recognized as a major source of food-borne diseases (EFSA/ECDC, 2014). STEC can cause several human gastrointestinal illnesses, including non-bloody or bloody diarrhea (Kaper et al., 2004; Karch et al., 2005). In a proportion of patients, commonly children, conditions may be complicated by neurologic and renal sequelae, including the life-threatening hemolytic-uremic syndrome (HUS) (Tarr et al., 2005). STEC are characterized by the production of one or more Shiga toxins: Stx1, Stx2, and subtypes (Johannes and Römer, 2010). STEC strains pathogenic for humans tend to produce Stx2 and have other virulence traits such as the adhesion factor intimin *(eae)* or *E. coli* enterohemolysin (*ehx*A) (Friedrich et al., 2002; Brooks et al., 2005; Orth et al., 2007b). Most outbreaks and sporadic cases of bloody diarrhea and HUS in humans have been attributed to STEC strains of serotype O157:H7, but the importance of non-O157 STEC (e. g. O26:H11/H$^-$, O91:H21/H$^-$, O103:H2, O111:H$^-$, O113:H21, O121:H19, O128:H2/H$^-$, or O145:H28/H$^-$) as causes of HUS, bloody diarrhea, and other intestinal illnesses is increasingly recognized (Johnson et al., 2006; Bettelheim, 2007; EFSA/ECDC, 2014).

Different isolation procedures have been proposed for STEC, but fast and reliable strain isolation, which is a prerequisite to assess the pathogenic traits, remains a challenge in routine diagnostics. Culture-based methods (e. g. sorbitol-MacConkey agar) are cost-effective options for STEC screening, but drawbacks such as false-negative results due to emerging serotypes of non-O157 STEC and sorbitol-fermenting O157:H$^-$ STEC limit the utility. The recently launched CHROMagar™ STEC medium (CHROMagar Microbiology, Paris, France) thereby might be a promising option for STEC screening under certain conditions (Hirvonen et al., 2012; Tzschoppe et al., 2012; Gouali et al., 2013; Wylie et al., 2013). Using this medium, STEC strains are expected to produce mauve colonies, which are either fluorescent (non-O157) or non-fluorescent (O157) under UV light. Growth on the CHROMagar™ STEC medium is associated with resistance to tellurite encoded by genes of the *ter* cluster (Hirvonen et al., 2012; Tzschoppe et al., 2012). Resistance to tellurite is common in STEC strains associated with (severe) human disease, but it is not as frequently found in other STEC isolates (Orth et al., 2007a; Tzschoppe et al., 2012). The aim of the present study was to evaluate the performance of the CHROMagar™ STEC medium by testing the growth capacity and the colony morphology of 74 target (STEC of various serotypes) and non-target bacterial strains.

Materials and methods

Bacterial strains used in this study comprised 39 STEC strains of various serotypes and 35 non-target strains including *stx*-negative *E. coli* isolates and non-*E. coli* isolates. The 39 STEC strains were isolated in the Swiss National Reference Center for Enteropathogenic Bacteria (NENT) from fecal samples of human patients with reasonable clinical suspicion of infection with STEC (Käppeli et al., 2011a/b). Characteristics of these strains and associated clinical symptoms are shown in Table 1. The 35 non-target strains were obtained from the collection of the Institute for Food Safety and Hygiene (Vetsuisse Faculty University of Zurich). Characteristics of the 28 non-target *E. coli* strains are shown in Table 2. The *stx*-negative/*eae*-positive *E. coli* strains mainly originated from fecal samples of healthy cattle at slaughter. The 13 *stx*-negative *E. coli* O157 strains (including two eae-positive isolates) originated from fecal samples of healthy cattle, sheep and pig at slaughter. The seven non-*E. coli* strains comprised isolates of the genus *Citrobacter, Klebsiella, Salmonella* and *Yersinia* (Table 3).

All bacterial strains were cultured from deep-frozen stokks (–80 °C) on sheep blood agar (Difco Columbia Blood Agar Base EH; Becton Dickinson AG, Allschwil, Switzerland; 5 % sheep blood SB055; Oxoid AG, Pratteln, Switzerland) for 24 h at 37 °C. One colony from each sheep blood agar plate was then streaked onto the CHROMagar™ STEC medium (CHROMagar Microbiology, Paris, France). After incubation for 24 h at 37 °C, the growth capacity and the phenotypic colony morphology (color formation) was evaluated.

Results and Discussion

Eighteen of the 39 STEC strains from human patients belonged to the top-five serogroups (five O26 strains, three O103 strains, one O111 strain, four O145 strains, five O157 strains). The majority (83.3%) of these 18 STEC strains did grow on the CHROMagar™ STEC medium and showed typical mauve colonies (Table 1). Growth was thereby observed for the O103, O111 and O145 STEC strains, whereas the O26:H34 strain and, as expected, the two

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sorbitol-fermenting, non-motile O157 strains could not grow. In other studies, growth on the CHROMagar™ STEC medium was closely associated with STEC strains belonging to serotypes of major importance in human disease (Hirvonen et al., 2012; Tzschoppe et al., 2012; Gouali et al., 2013; Wylie et al., 2013; Gill et al., 2014), but it must be considered that a certain proportion of them also failed to grow. Inconsistent results were thereby obtained in particular for STEC of serotype O103:H2 (Hirvonen et al., 2012; Tzschoppe et al., 2012; Gill et al., 2014).

Of the remaining 21 STEC strains (non-top-five serogroups) from human patients, only five strains of

TABLE 1: *Characteristics of the 39 STEC strains isolated from human patients and their growth properties on CHROMagar™ STEC*[a].

Serotype	Strain ID	Growth on CHROMagar™ STEC (color of colonies)	Sorbitol	*stx*1	*stx*2	*eae*	*ehx*A	Disease
O8:H16	645-01	**No growth**	SF	+	+	–	+	BD
O20:H30	1402-02	**No growth**	SF	+	–	–	–	HUS
O26:[H11]	1972-08	+ (mauve)	SF	+	+	+	+	A, D, HUS
O26:H11	3528-00	+ (mauve)	SF	–	+	+	+	HUS
O26:H11	1183-02	+ (mauve)	SF	+	+	+	+	D, HUS
O26:H11	1614-08	+ (mauve)	SF	–	+	+	+	BD, HUS
O26:H34	1973-08	**No growth**	SF	–	+	+	–	D, HUS
O55:H7	1976-08	**No growth**	NSF	+	–	+	–	A, D, HUS
O82:H8	2329-08	**No growth**	SF	+	+	–	+	A, BD, HUS
O91:H10	1463-03	**No growth**	SF	–	+	–	–	BD, HUS
O98:[H21]	878-03	+ (mauve)	SF	+	–	+	+	BD
O103:[H2]	756-07	+ (mauve)	SF	+	–	+	+	BD
O103:H2	1477-07	+ (mauve)	SF	+	–	+	+	D
O103:H2	2249-08	+ (mauve)	SF	+	–	+	+	BD
O105:H18	1805-06	**No growth**	NSF	+	+	–	+	BD
O111:[H8]	1086-00	+ (mauve)	SF	+	–	+	+	BD
O113:H4	968-03	**No growth**	SF	+	+	–	+	BD
O117:H7	117-00	**No growth**	NSF	+	–	–	–	BD
O121:H19	1850-04	+ (mauve)	SF	–	+	+	+	HUS
O123:H2	130-03	+ (mauve)	SF	+	–	+	+	BD
O145:H25	995-07	+ (mauve)	SF	–	+	+	+	A, D, HUS
O145:[H25]	1550-08	+ (mauve)	SF	–	+	+	+	A, HUS
O145:[H28]	383-04	+ (mauve)	SF	–	+	+	+	BD
O145:[H28]	1094-07	+ (mauve)	SF	–	+	+	+	A, BD
O148:H8	128-03	**No growth**	SF	–	+	–	–	A, HUS
O153:[H4]	1146-05	**No growth**	SF	+	–	–	+	HUS
O157:H7	154-00	+ (mauve)	NSF	–	+	+	+	ND
O157:H7	1527-00	+ (mauve)	NSF	–	+	+	+	HUS
O157:H7	2965-00	+ (mauve)	NSF	–	+	+	+	BD
O157:[H7]	002-04	**No growth**	SF	–	+	+	+	BD
O157:[H7]	774-04	**No growth**	SF	–	+	+	+	D, HUS
O167:H32	1933-06	**No growth**	SF	+	–	–	–	BD
O174:H2	1533-01	+ (mauve)	SF	+	+	–	+	BD
O177:[H25]	908-02	+ (white)	NSF	–	+	+	+	BD
O181:H49	1237-04	**No growth**	SF	+	+	–	+	HUS
Or:H7	012-06	**No growth**	NSF	+	–	–	–	BD
Or:[H25]	2841-01	**No growth**	NSF	–	+	+	+	HUS
Or:H31	1325-02	**No growth**	SF	–	+	–	–	BD
Or:H-	1677-05	+ (mauve)	SF	+	–	+	+	BD

[a]: STEC, Shiga toxin-producing *Escherichia coli*; SF, sorbitol-fermenting; NSF, non-sorbitol-fermenting *stx*, Shiga toxin gene; *eae*, intimin gene; *ehx*A, enterohemolysin gene; Serotypes in bold indicate STEC belonging to the top-five serogroups (O26, O103, O111, O145, O157); Or, O rough; +, positive; –, negative; [ ], non-motile strains investigated with respect to their flagellar genotypes by PCR; A, anemia; BD, bloody diarrhea; D, non-bloody diarrhea; HUS, hemolytic-uremic syndrome; ND, no data.

TABLE 2: *Characteristics of the 28 non-target E. coli strains and their growth properties on CHROMagar™ STEC[a].*

Organism	Strain ID	Growth on CHROMagar™ STEC (color of colonies)	*stx*	*eae*	Origin
E. coli O2:H45	4286/1	+ (mauve)	–	+	Cattle
E. coli O26	36	+ (mauve)	–	+	Cattle
E. coli O26	5	+ (mauve)	–	+	Cattle
E. coli O26	321	+ (mauve)	–	+	Cattle
E. coli O26	222	+ (mauve)	–	+	Cattle
E. coli O26	3733	+ (mauve)	–	+	Cattle
E. coli O103:H2	4215/4	No growth	–	+	Cattle
E. coli O103:H4	2315-11	No growth	–	+	Human
E. coli O103:H8	4075/1	No growth	–	+	Cattle
E. coli O111:H25	1120-10	No growth	–	+	Human
E. coli O113:H6	3830/2	+ (mauve)	–	+	Cattle
E. coli O121:H45	2211-10	No growth	–	+	Human
E. coli O128:H2	3970/4	+ (mauve)	–	+	Cattle
E. coli O145:H28	1119-10	+ (mauve)	–	+	Human
E. coli O177:H11	4289/1	+ (mauve)	–	+	Cattle
E. coli O157:H2	7770/1	No growth	–	–	Cattle
E. coli O157:H7	2905	No growth	–	–	Cattle
E. coli O157:H7	396	No growth	–	–	Sheep
E. coli O157:H8	999	No growth	–	–	Cattle
E. coli O157:H12	264	No growth	–	–	Sheep
E. coli O157:H18	332	No growth	–	–	Pig
E. coli O157:H19	2929	No growth	–	–	Cattle
E. coli O157:H25	419	No growth	–	–	Cattle
E. coli O157:H45	584	No growth	–	+	Pig
E. coli O157:H27	877	No growth	–	–	Cattle
E. coli O157:H38	261	No growth	–	–	Cattle
E. coli O157:H43	740	No growth	–	–	Cattle
E. coli O157:H45	922	No growth	–	+	Cattle

[a]: *stx*, Shiga toxin gene; *eae*, intimin gene; +, positive; –, negative.

serogroups O98, O121, O123, O174 and O rough did grow on the CHROMagar™ STEC medium and showed typical mauve colonies (Table 1). The O177 strain also showed growth, but colonies were of white color. Thus, the majority (76.2 %) of these 21 STEC strains belonging to a variety of serogroups could not grow on the CHROMagar™ STEC medium (including e. g. O55, O91 or O113 isolates). Non-growth of such STEC strains is common (Hirvonen et al., 2012; Tzschoppe et al., 2012; Gill et al., 2014) and seems not to be serotype-specific (Wylie et al., 2013). It must be emphasized that the STEC strains included in the present study were mainly isolated from human patients suffering from severe disease conditions (Table 1) and also certain STEC strains not-belonging to the top-five serogroups (e.g. O91, O113, O121 or 128 isolates) might play an important role as a cause of human disease in certain geographical regions (Beutin et al., 2004; Brooks et al., 2005; Bettelheim, 2007). Moreover, the use of CHROMagar™ STEC as the only medium for STEC screening or prevalence studies will result in the selection of certain STEC strains and comparison with surveys applying other methods are questionable.

The 35 non-target strains investigated in the present study included 28 *stx*-negative *E. coli* strains (Table 2). The majority of the *stx*-negative/*eae*-positive *E. coli* strains could grow on the CHROMagar™ STEC medium and showed typical mauve colonies. The 10 *stx*-negative/*eae*-positive *E. coli* strains with growth capacity belonged to the serogroups O2, O26, O113, O128, O145 and O177. Growth of various *stx*-negative/*eae*-positive *E. coli* strains on the CHROMagar™ STEC medium has also been reported by Gouali et al. (2013). Thereby, it must be considered that such *E. coli* strains, in particular *E. coli* O26 and O157 isolates (Bielaszewska et al., 2007; Mellmann et al., 2008), can undergo ephemeral interconversions via loss and gain of Stx-encoding phages. On the other hand, the CHROMagar™ STEC medium inhibited successfully the growth of *stx*-negative/*eae*-positive *E. coli* O103, O111 and O121 strains and of all *stx*-negative *E. coli* O157 strains. Similarly, none of the investigated non-*E. coli* strains *(Citrobacter, Klebsiella pneumonia, Salmonella, Yersinia enterocolitica)* showed growth on the CHROMagar™ STEC medium (Table 3). Various non-*E. coli* strains have been tested for their growth on the CHROMagar™ STEC medium in other studies, but these strains could not grow or colonies were not mauve (Hirvonen et al., 2012; Teramura et al., 2013).

In conclusion, most of the STEC strains belonging to the top-five serogroups (O26, O103, O111, O145, O157) did grow on the CHROMagar™ STEC medium and showed typical mauve colonies. However, the majority of STEC strains not belonging to the top-five serogroups could not grow, whereas a variety of *stx*-negative/*eae*-positive *E. coli* strains had the capacity to grow on this medium. Thus, CHROMagar™ STEC medium can not be recommended as primary STEC screening method in routine diagnostics. This medium should therefore only be used for specific questions (e. g. the isolation of STEC strains belonging to the top-five serogroups) or in routine STEC diagnostics in combination with another method or medium.

TABLE 3: *Growth of selected non-E. coli strains on the CHROMagar™ STEC medium (n = 7).*

Organism	Strain ID	Growth on CHROMagar™ STEC (color of colonies)
Citrobacter	C778	No growth
Citrobacter	C1102	No growth
Klebsiella pneumoniae	220_K	No growth
Salmonella	1935-13	No growth
Salmonella	1938-13	No growth
Yersinia enterocolitica	1565-13	No growth
Yersinia enterocolitica	1566-13	No growth

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Address of corresponding author:
Prof. Dr. Roger Stephan
Institute for Food Safety and Hygiene
Vetsuisse Faculty University of Zurich
Winterthurerstrasse 272
CH-8057 Zurich
Tel.: +41-44-635-8651; Fax: +41-44-635-8908
stephanr@fsafety.uzh.ch