Towards a Molecular Definition of Enterohemorrhagic Escherichia coli (EHEC): Detection of Genes Located on O Island 57 as Markers To Distinguish EHEC from Closely Related Enteropathogenic E. coli Strains

Sabine Delannoy, a Lothar Beutin, b Patrick Fach a
Anses (French Agency for Food, Environmental, and Occupational Health and Safety), Food Safety Laboratory, Maisons-Alfort, France; a National Reference Laboratory for Escherichia coli, Division of Microbial Toxins, Federal Institute for Risk Assessment (BfR), Berlin, Germany

Among strains of Shiga-toxin (Stx) producing Escherichia coli (STEC), seven serogroups (O26, O45, O103, O111, O121, O145, and O157) are associated with severe clinical illness in humans. These strains are also called enterohemorrhagic E. coli (EHEC), and the development of methods for their reliable detection from food has been challenging thus far. PCR detection of major EHEC virulence genes stx1, stx2, eae, and O-serogroup–specific genes is useful but does not identify EHEC strains specifically. Searching for the presence of additional genes issued from E. coli O157:H7 genomic islands OI-122 and OI-71 increases the specificity but does not clearly discriminate EHEC from enteropathogenic E. coli (EPEC) strains. Here, we identified two putative genes, called Z2098 and Z2099, from the genomic island OI-57 that were closely associated with EHEC and their stx-negative derivative strains (87% for Z2098 and 91% for Z2099). Z2098 and Z2099 were rarely found in EPEC (10% for Z2098 and 12% for Z2099), STEC (2% and 15%), and apathogenic E. coli (1% each) strains. Our findings indicate that Z2098 and Z2099 are useful genetic markers for a more targeted diagnosis of typical EHEC and new emerging EHEC strains.

Enterohemorrhagic Escherichia coli (EHEC) as a subgroup of Shiga-toxin (Stx)-producing E. coli (STEC) are characterized by certain serotypes that are frequently occurring in outbreaks and are associated with severe clinical illnesses such as hemolytic-uremic syndrome (HUS) (1, 2). In the developed countries of the Northern Hemisphere, serotypes O26: H11, O45:H2, O103:H2, O111:H8, O121:H19, O145:H28, O157: H7, and their nonmotile derivatives are the seven “priority” STEC serotypes (referred to as the top7 serotypes) most frequently implicated in outbreaks and sporadic cases of HC and HUS (3–6). These “priority” STEC have been categorized into seropathotypes (SPTs) A and B based on their phenotypic and molecular characteristics and the clinical features of the associated diseases. SPTs range from A to E, according to a decreasing rank of pathogenicity.

Besides the ability to produce one or more types of Shiga toxins, the top7 EHEC strains harbor a genomic island, called the locus of enterocyte effacement (LEE), encoding the intimin (eae gene), which participates in bacterial colonization of the gut and in attaching-and-effacing (A/E) lesions of the intestinal mucosa (2). The LEE also encodes regulatory elements, a type III secretion system, secreted effector proteins, and their cognate chaperon (7, 8). Currently, standard methods use stx, eae, and O-serogroup–specific gene sequences for detection of the top7 EHEC serotype strains (9, 10). However, detection of these targets in food and stool enrichment broths does not necessarily indicate the presence of a particular EHEC strain as the eae gene is also present in Stx-negative enteropathogenic E. coli (EPEC) strains (2) that may be present in food and stool samples. For food inspection laboratories, a genetic marker allowing a clear distinction between EHEC and EPEC strains before isolation of bacteria is of great value for timely analysis of samples. Ideally, such a marker could be integrated into screening methods for the detection of “priority” STEC posing a great risk to public health.

Molecular risk assessment approaches based on the assessment of the virulence gene content derived from a number of pathogenicity islands have been used to predict whether an STEC strain might pose a significant risk to human health (11–16). A variable repertoire of virulence determinants, including a panel of non-LEE-encoded effector (nle) genes that encode translocated substrates of the type III secretion system have been identified in EHEC strains (11–14, 16, 17). Six different nle genes issued from genomic Island 71 (ent/espL2, nleB, and nleE) and O Island 71 (nleF, nleH1-2, and nleA) have been tested for their prevalence in STEC, EHEC, EPEC, and nonpathogenic E. coli strains (13, 14). The presence of eae, ent/espL2, nleB, nleE, and nleH1-2 genes proved to be a clear signature of recognized EHEC and new emerging EHEC types such as O5:HND, O15:H2, O118:H16, and O103:H25. However, since these nle genes are also frequent in some EPEC strains (16), they are not suitable to be used as predictive genetic markers for monitoring EHEC in food.

A recent study pointed out that genes carried by the genomic O island 57 (O1-57) may be associated with increased virulence of STEC strains to humans (18). These include the adfO gene (pro-

Received 25 October 2012 Returned to author 10 December 2012 Accepted 11 January 2013Published ahead of print 16 January 2013Address correspondence to Patrick Fach, patrick.fach@anses.fr.Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.02864-12.Copyright © 2013, American Society for Microbiology. All Rights Reserved.
doi:10.1128/JCM.02864-12
motivating adherence of EHEC to in vitro–cultured cells) (19) and the ckf gene, encoding a putative phage-associated killer protein. Paralogues of this protein have been reported to disrupt the bacterial host membranes when produced in excess (20). It has been reported that adfO is present, together with ckf in 92.7% of human EHEC strains belonging to SPTs A and B, whereas both genes are absent in STEC strains not associated with disease (SPTs D and E) and in apathogenic E. coli strains (18). However, both genes are present in 71% of EPEC strains as well (18). Interestingly, O1-57 has also been shown to carry other open reading frames (ORFs), such as Z2097, Z2098, Z2121, and Z2149, which seemed to be associated preferentially with the EHEC strains according to Imamovic et al. (18).

Analysis of O1-57-encoded genes in EPEC revealed a higher degree of variability than observed in STEC, with <25% of the strains carrying the complete set of O1-57 ORFs tested by Imamovic et al. (18). These findings prompted us to investigate the relationship between EHEC and EPEC strains for certain ORFs encoded by the O1-57 and in a second step to explore the suitability of certain O1-57 genes as markers to discriminate between EHEC and EPEC strains.

We identified two ORFs, Z2098 and Z2099, as suitable genetic markers for identification of human virulent STEC strains and for EHEC and EPEC strains. The STEC strains included eae-negative E. coli of serotypes O91: [H21], O113:[H21], and O104:[H21], which are categorized into SPT C. STEC isolates of SPT C were reported to be less frequently involved in HC and HUS than SPT A and B strains but are known to be a common cause of diarrhea in humans (2). Stx-negative derivatives of EHEC strains belonging to the top7 serotypes were designated as EHEC-like. These were defined on the basis of their nle gene profiles, their eae subtypes, and their serotypes (13, 16). EHEC-like strains of serotype O26:H11 were also identified by the presence of the espK gene and the allelic type 2 of the arcA housekeeping gene (15). EPEC strains were defined as described by Bugarel et al. (16).

All of the strains investigated here were identified for the E. coli O (lipopolysaccharide) and H (flagellar) antigens and have been characterized for the stx and eae genes as previously reported (14). For examination, bacteria were cultured to single colonies on Luria broth plates and grown overnight at 37°C. One colony was picked up and DNA extracted using the InstaGene matrix (Bio-Rad Laboratories, Marnes-La-Coquette, France) before high-throughput real-time PCR testing.

**MATERIALS AND METHODS**

**Bacterial strains.** The E. coli strains investigated in the present study were obtained from the collection of the National Reference Laboratory for E. coli at the Federal Institute for Risk Assessment (BfR) in Berlin, Germany, and from the French Agency for Food, Environmental, and Occupational Health and Safety (Anses) in Maisons-Alfort, France (see Table S1 in the supplemental material). The E. coli strains used here were largely derived from the panel of strains used in previous studies (13, 14, 16, 21, 22). The strains of E. coli (n = 1100) were grouped into EHEC and EHEC-like strains (EHEC strains and their stx-negative derivatives) (n = 413), EPEC strains (enteropathogenic E. coli) (n = 322), STEC strains (Shiga-toxin producing E. coli) (n = 190), and apathogenic E. coli strains (n = 175) according to the presence or absence of genes encoding Stx (stx1, stx2) and intimin (eae). EHEC strains were defined as those harboring both a stx (stx1 and/or stx2) and eae gene. EPEC strains harbor eae only and STEC only stx. Apathogenic E. coli were designated as E. coli strains that do not possess stx and eae and which were isolated from feces of humans without enteric disease. We cannot exclude that these strains carry other virulence genes involved in extraintestinal infections of humans or cause diarrhea by other mechanisms than EPEC or STEC.

The STEC strains included eae-negative E. coli of serotypes O91: [H21], O113:[H21], and O104:[H21], which are categorized into SPT C. STEC isolates of SPT C were reported to be less frequently involved in HC and HUS than SPT A and B strains but are known to be a common cause of diarrhea in humans (2). Stx-negative derivatives of EHEC strains belonging to the top7 serotypes were designated as EHEC-like. These were defined on the basis of their nle gene profiles, their eae subtypes, and their serotypes (13, 16). EHEC-like strains of serotype O26:H11 were also identified by the presence of the espK gene and the allelic type 2 of the arcA housekeeping gene (15). EPEC strains were defined as described by Bugarel et al. (16).

All of the strains investigated here were identified for the E. coli O (lipopolysaccharide) and H (flagellar) antigens and have been characterized for the stx and eae genes as previously reported (14). For examination, bacteria were cultured to single colonies on Luria broth plates and grown overnight at 37°C. One colony was picked up and DNA extracted using the InstaGene matrix (Bio-Rad Laboratories, Marnes-La-Coquette, France) before high-throughput real-time PCR testing.

**High-throughput real-time PCR.** A LightCycler 1536 (Roche, Meylan, France) was used to perform high-throughput real-time PCR amplifications. For the PCR setup of the LightCycler 1536 multivell plate, a Bravo liquid dispenser automat (Agilent Technologies, Massy, France) equipped with a chiller and a PlateLoc thermal microplate sealer (Agilent Technologies) was used. The PCRs contained 0.5 μl of DNA sample and 1 μl of Master mix containing 1 × RealTime ready DNA Probes Master Roche (corresponding to ×0.7 final), 300 nM concentrations of each primer, and 300 nM concentrations of each probe (corresponding to 200 nM [final] for each). Amplifications were performed using FAM- or HEX-labeled TaqMan probes. The following thermal profile was used for PCR: 95°C for 1 min, followed by 35 cycles of 95°C for 0 s and 60°C for 30 s, with a final cooling step at 40°C for 30 s. The genes stx1, stx2, eae, and nleB were used as internal controls and for group assignment purposes. The primers and probes for the detection of stx1, stx2, eae, and nleB genes were described previously (13, 14). Primers and probes for the detection of Z2098, Z2099, and Z2121 were designed for the present study and are listed in Table 1.

**RESULTS**

Association of the putative O1-57 encoded genes Z2098, Z2099, and Z2121 with EHEC and EHEC-like strains. We analyzed 1,100 E. coli strains, including EHEC and EHEC-like strains (n = 413), EPEC strains (n = 322), and STEC strains (n = 190), as well as

### Table 1: Primers and probes for real-time PCR detection of OI-57

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primer or probe</th>
<th>Sequence (5’–3’)*</th>
<th>Location within sequence AE005174*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z2098</td>
<td>Forward primer</td>
<td>CTGAAAAAGCCAGAGCGTGC</td>
<td>1888173–1888193</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>TGCCCTAGATCATGACGGACG</td>
<td>1888308–1888287</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>[HEX]TAATGTCATACCTCAGGGCGGCGG[BAQ]</td>
<td>1888285–1888265</td>
</tr>
<tr>
<td>Z2099</td>
<td>Forward primer</td>
<td>TAGCGGGACAGATTTGACGGG</td>
<td>1889124–1889143</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>GTCTTTCGGAGAGATGACGG</td>
<td>1889190–1889168</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>[HEX]ATATTGATGACGGAATGGTATGAGGCGG[BAQ]</td>
<td>1889144–1889167</td>
</tr>
<tr>
<td>Z2121</td>
<td>Forward primer</td>
<td>GATGGCAGATATACGAGGAGCAAC</td>
<td>1904798–1904821</td>
</tr>
<tr>
<td></td>
<td>Reverse primer</td>
<td>CAGCCGTTGAAAGCTGACGG</td>
<td>1904915–1904896</td>
</tr>
<tr>
<td></td>
<td>Probe</td>
<td>[HEX]AGAGGCTTTGTCAGACGCTTTACGG[BAQ]</td>
<td>1904823–1904848</td>
</tr>
</tbody>
</table>

* HEX, hexachlorofluorescein; BAQ, Black Hole Quencher.

**Numbering as in EDL933.**
nonpathogenic *E. coli* strains (*n* = 175), for the presence of the three putative genes Z2098, Z2099, and Z2121 encoded by the OI-57 genomic island. The distribution of these genetic markers in different *E. coli* (patho)groups is shown in Fig. 1. Genetic markers Z2098 and Z2099 were frequently detected in EHEC and EHEC-like strains (87% for Z2098 and 91% for Z2099). In contrast, they were rarely found in EPEC strains (10% for Z2098 and 12% for Z2099), STEC strains (2 and 15%, respectively), and apathogenic *E. coli* strains (1% each). Z2121 was significantly associated with EHEC, EHEC-like, and EPEC strains (*P* < 0.001), since it is present in 94% of EHEC and EHEC-like strains and also in 58% of EPEC strains. Z2121 was detected in only 1% of the STEC strains and 2% of the apathogenic *E. coli* strains.

The distribution of Z2098 and Z2099 among strains belonging to the top7 EHEC serotypes is not uniform (Table 2). Z2098 was detected in almost all EHEC and EHEC-like strains of serotypes O103:H2, O145:H28, O111:H8, O26:H11, O121:H19, and O45:H2 (92.5%) but less frequently in serotype O157:H7 (77.3%) and in new emerging EHEC strains (76.1%). Z2099 was detected in almost all EHEC and EHEC-like serotypes O103:H2, O145:H28, O111:H8, O26:H11, O121:H19, and O45:H2 (92.9%) and in new emerging EHEC strains (91.0%), although at different frequencies. Z2099 was less common in serotype O121:H19 (72.0%), O45:H2 (82.4%), and O157:H7 (85.3%) strains than in serotype O103:H2 (93.55%), O111:H8 (94.23%), O26:H11 (97.4%), and O145:H28 (100%) strains (Table 2). Interestingly, if we consider strictly the association of the presence of *stx*, *eae*, and Z2099 in these serogroups, it is remarkable that Z2099 was detected in 100% of EHEC O103:H2, O26:H11 and O145:H28 strains, showing a close relationship between the presence of Z2099 with

### TABLE 2 Distribution of Z2098 and Z2099 in EHEC and EHEC-like strains

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Presence (%) in EHEC strains&lt;sup&gt;a&lt;/sup&gt;</th>
<th>New emerging EHEC strains&lt;sup&gt;d&lt;/sup&gt; (n = 67)</th>
<th>Genes absent in EHEC and EHEC-like strains&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z2099</td>
<td>85.33 (78.61–92.05)</td>
<td>92.99 (90.44–95.54)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>O103:H2 (n = 4)<em>, O111:H8 (n = 3)</em>, O121:H19 (n = 3)<em>, O121:H19 (n = 4)</em>, O127:H8s*, O157:H7 (n = 11)<em>, O26:H11 (n = 2)</em>, O3*, O45:H2 (n = 3)<em>, O55:H7 (n = 2)</em>, O76:H51*, Or:H16*</td>
</tr>
</tbody>
</table>

<sup>a</sup>The 95% confidence intervals are indicated in parentheses.

<sup>b</sup>* EHEC strains; †, EHEC-like strains. For each serotype, *n* = 1 unless otherwise stated. Values for strains from the top7 EHEC serotypes are indicated in boldface.

<sup>c</sup>The top6 EHEC strains include EHEC strains belonging to serotypes O103:H2, O145:H28, O111:H8, O26:H11, O121:H19, and O45:H2 and their *stx*-negative derivatives.

<sup>d</sup>The new emerging EHEC strains include all EHEC serotypes different from O157:H7 and EHEC top6 strains and their *stx*-negative derivatives.

<sup>e</sup>Values for the individual serotypes: O121:H19, 72.0% (18/25); O103:H2, 93.6% (58/62); O111:H8, 94.2% (49/52); O26:H11, 97.4% (75/77); O45:H2, 82.4% (14/17); and O145:H28, 100% (38/38).
the virulence factors (stx and eae) in these particular EHEC serotypes.

EHEC and EHEC-like strains negative for Z2098 and/or Z2099 are listed in Table 2. The 36 strains that were negative for Z2099 comprised 26 EHEC strains of serotypes O157:H7 (n = 11), O111:H8 (n = 3), O121:H19 (n = 3), O127:H8 (n = 1), O3 (n = 1), O45:H2 (n = 3), O55:H7 (n = 2), O76:H51 (n = 1), and Or:H16 (n = 1), as well as 10 EHEC-like strains belonging to serotypes O103:H2 (n = 4), O121:H19 (n = 4), and O26:H11 (n = 2). EHEC and EHEC-like strains negative for Z2098 comprised the 26 Z2099-negative strains, 1 EHEC-like strain that belonged to serotype O121:H19, and 17 EHEC strains of serotypes O111:H8 (n = 1), O123:H11 (n = 1), O156:H21 (n = 1), O157:H7 (n = 6), O165:H25 (n = 1), O172:H125 (n = 2), O172:HNM (n = 1), O49:H16 (n = 1), Ont:H2 (n = 1), Ont:H25 (n = 1), and O186: [H2] (n = 1).

Presence of Z2098 and Z2099 in EPEC, STEC, and apathogenic E. coli strains. The EPEC, STEC, and apathogenic E. coli strains giving a positive reaction with Z2098 and/or Z2099 are reported in Table 3. Among the 190 STEC strains tested, 4 (2%) were positive for Z2098, and 29 (15%) were positive for Z2099. Among the positive strains were some human virulent STEC strains belonging to SPT C (O104:H21, O146:H21 and O91). Thirty-two (10%) of the 322 EPEC strains tested were positive for Z2098, and 38 (12%) were positive for Z2099 (Table 3). The genetic markers Z2098 and Z2099 were also present in 1% of each of the nonpathogenic E. coli strains tested, including serotypes O110 and O132:H18 (Table 3).

Diagnostic application of Z2098 and Z2099 in the detection of typical EHEC strains. A collection of 1,100 strains originating from humans, animals, and food were used to assess the efficacy of real-time PCR assays targeting the genes Z2098 and Z2099 that were developed in the present study. The relative-sensitivity estimate of the assays was calculated as the ratio of the number of EHEC and EHEC-like positive (true positive) versus the total number of EHEC and EHEC-like strains that tested negative (true negative) versus the total number of non-EHEC and non-EHEC-like strains. As a whole, the Z2098-PCR assay used for the detection of EHEC and EHEC-like strains showed a relative-sensitivity estimate of 86.9% and a relative-specificity estimate of 94.5%. The real-time PCR assay targeting the gene Z2099 showed a higher relative-sensitivity estimate of 91.3% and a relative-specificity estimate of 90.0%.

**DISCUSSION**

The emergence of O157 and non-O157 EHEC strains in severe and epidemic human disease is of global concern (3–5, 9). Current methods for the detection of EHEC in food samples are based on real-time PCR detection of stx and eae genes, followed by the detection of EHEC-associated O-antigen genes (O26, O45, O103, O111, O121, O145, and O157) (9, 10). A problem is that many STEC, EPEC, and apathogenic E. coli strains can also react with these genetic targets. This is of particular interest if samples from food, feces, and the environment are examined. Genetic markers allowing more specifically the identification of EHEC strains (in particular those of the top7 EHEC serotypes) and giving no cross-reactivity with EPEC strains are needed for more targeted investigations of these samples.

With the objective to identify genetic markers representative for EHEC strains, several genes carried by the O island 122 (OI-122), O island 71 (OI-71), and O island 57 (OI-57) have been tested by PCR (11–16, 18). Since the genes identified on these genomic islands are also found in many EPEC strains, the search for an EHEC specific genetic target is still challenging. The genetic composition of the island OI-57 has been investigated previously by PCR amplification of OI-57-associated ORFs, indicating differences between EHEC and EPEC strains (16, 18). Some OI-57-encoded genes were found more closely associated with EHEC strains, but the number of strains investigated in these studies was too small to draw conclusions about the significance of this finding (18).

Our study aimed to further examine the genomic island OI-57 and to test its ability to carry discriminatory diagnostic targets for identifying with high specificity and sensitivity strains of the top7 EHEC serotypes and new emerging EHEC strains. We explored

---

**TABLE 3 Detection of Z2098 and Z2099 in EPEC, STEC and apathogenic E. coli strains**

<table>
<thead>
<tr>
<th>Strain type</th>
<th>Serotypes</th>
<th>n</th>
<th>Serotypes</th>
<th>n</th>
<th>Serotypes</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEC strains</td>
<td>O104:H7, O110, O117, O146:H8</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apathogenic E. coli strains</td>
<td>O110, O132:H18</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*For each serotype, n = 1 unless otherwise stated.

This list includes STEC strains from SPT C (indicated in boldface).
usint BLAST-N the ORFs of the OI-57 and identified by in silico analysis Z2098, Z2099, and Z2121 as potentially specific genetic markers of EHEC and their stx-negative derivatives. High-throughput real-time PCR was used to study the distribution of these three genetic markers localized on the OI-57 in a representative collection of EHEC (n = 413), EPEC (n = 322), STEC (n = 190), and nonpathogenic E. coli (n = 175) strains from different origins, sources, and time periods. We identified genes Z2098 and Z2099 as suitable candidates for searching EHEC more specifically, since these genes were significantly more prevalent in EHEC strains and their stx-negative derivatives (87 to 91%) than in the other E. coli (patho)groups (1 to 15%). Since the third genetic marker, Z2121, was also frequent in EPEC strains (58%), it is not useful for EHEC screening.

It is noteworthy that most of the strains of the top7 EHEC O serogroups but with H types different from those of top7 EHEC strains also tested negative for Z2098 and Z2099. Among these were some STEC O91, O104, and O113 (SPT C) and STEC O174 and O146, which are known to be associated with bloody diarrhea in humans. These strains were previously called atypical EHEC because they lack the LEE genes encoding the A/E phenotype (2). Searching for Z2098 and/or Z2099 might be useful for detecting such atypical EHEC strains that are not detected by current detection assays determining the presence of stx and eae genes (9,10).

In addition, our study pointed out some EPEC strains that tested positive for Z2098 and/or Z2099. These were mostly atypical EPEC strains that resemble EHEC and are closely associated with outbreaks of diarrhea (16). The presence of Z2098 and Z2099 in certain SPT C strains and atypical EPEC strains resembling EHEC points to the association of Z2098 and Z2099 with E. coli strains implicated in severe clinical illness in humans. Further studies need to be conducted to substantiate this hypothesis.

Overall, Z2099 appears to be the best candidate to detect non-O157 EHEC strains of serotypes O26:H11, O45:H2, O103:H2, O111:H8, O121:H19, and O145:H28 and their nonmotile derivatives. If we consider strictly the association of the presence of stx, eae, and Z2099 in E. coli O103:H2, O26:H11, and O145:H28, it is remarkable that Z2099 was detected in 100% of the tested strains (data not shown). This finding shows the strong correlation of the presence of Z2099 with the virulence factors (stx and eae) for these particular EHEC serotypes. Z2099 reacts also with new emerging EHEC (O103:H25, O118:H16, O118:H2, O119:H25, O123:H11, O145, O156:H21, O156:H25, O165:H25, O172:H25, O172:H25, O177:H25, O182:H25, O49:H16, O5, O84:H2, O14:H2, O145:H25, and O146:H2). Z2099 offers a better relative-sensitivity estimate than Z2098 with a comparable relative-specificity estimate. Z2099 codes for a hypothetical 12-kDa protein that contains a bacterial OB-fold (BOF) motif, which is thought to play a role in bacterial pathogenicity (23).

In recent studies we introduced the CRISPR sequences as suitable genetic markers for specific identification of strains belonging to the top7 EHEC serotypes (22). However, this approach necessitates performing eight different PCR assays for assignment of strains to the top7 EHEC serotypes. Advantageously, PCR detection of Z2099 as a unique genetic target highly representative of EHEC could be used prior to CRISPR typing. A complete evaluation of such an approach on spiked and naturally contaminated samples will be crucial to definitely know how the PCR assay targeting Z2099 could be included in a strategy of rapid screening of the seven “priority” STEC serotypes along the food chain.

ACKNOWLEDGMENTS

We are grateful to F. Scheutz (WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella, Copenhagen, Denmark), A. Gill (Health Canada, Ottawa, Canada), M. Rivas (INEL-ANLIS, Buenos Aires, Argentina), G. H. Loneragan (Texas Tech University, Lubbock, TX), and C. DeRoy (The Pennsylvania State University, State College, PA), Roxane M. F. Piazza (Instituto Butantan, Sao Paulo, Brazil), and Peter Feng (U.S. Food and Drug Administration, College Park, MD) for providing E. coli isolates or DNA extracts from E. coli.

REFERENCES

15. Bugard M, Beutin L, Schuetz F, Loukiadis E, Fuch P. 2011. Identifica-


