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Isolation and Characterization of a β-D-Glucuronidase-Producing Strain of *Escherichia coli* Serotype O157:H7 in the United States

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A phenotypic variant of *Escherichia coli* serotype O157:H7 (G5101) was isolated from a patient with bloody diarrhea. Strain G5101 does not ferment sorbitol but is β-D-glucuronidase and urease positive. Serotyping and colony hybridization using a serotype-specific DNA probe confirmed that the isolate was O157:H7. G5101 produces Shiga-like toxins I and II and contains an *eae* gene that is highly conserved in the O157:H7 serotype. This strain would have been missed by laboratories that screen for the sorbitol-negative, β-D-glucuronidase-negative phenotype in isolating *E. coli* O157:H7 from clinical and food specimens.

Escherichia coli of serotype O157:H7 was first recognized as a cause of bloody diarrhea in humans in 1982, when two outbreaks in Michigan and Oregon were traced to ground beef contaminated with this organism (10). Isolates of O157:H7 and an occasional nonmotile variant belong to a pathogenic group known as enterohemorrhagic *E. coli* (EHEC). The pathogenicity of EHEC appears to be associated with a number of virulence factors that include attaching and effacing factors and the production of several cytotoxins. The cytotoxins (Shiga-like toxin I [SLT-I], SLT-II, and variants of SLT-II) are collectively known as SLTs because SLT-I is almost identical to the Shiga toxin of *Shigella dysenteriae* type 1. Although other *E. coli* serotypes share these virulence factors and have also been associated with human illness (4), *E. coli* O157:H7 remains the most frequent cause of hemorrhagic colitis, which may progress to the life-threatening hemolytic-uremic syndrome (HUS).

Isolates of serotype O157:H7 and the nonmotile variant group cluster in a few closely related enzyme types by multilocus enzyme electrophoresis and are more distantly related to other *E. coli* isolates (13). This high degree of genetic homogeneity has provided some unique metabolic phenotypes, which have facilitated the isolation and identification of serotype O157:H7 from food, clinical, and environmental specimens. Unlike other *E. coli* isolates, *E. coli* O157:H7 isolates do not ferment sorbitol in 24 h; hence, differential selection on MacConkey agar containing sorbitol has been very effective in isolating this pathogen from bloody stool specimens (4). Serotype O157:H7 also does not exhibit β-glucuronidase (GUD) activity (11); therefore, many laboratories, particularly those involved in food analysis, further screen sorbitol-negative colonies for GUD (7, 12). GUD assays are done easily by incorporating commercially available fluorogenic or colorimetric substrates in routine culture media. In their analysis of ground beef samples, Okrend et al. (7) reported that further screening of sorbitol-negative colonies for GUD reduced the number of false-positive identifications by 36% from that found by selection solely on the basis of the sorbitol phenotype.

We report the first isolation in the United States of an atypical O157:H7 isolate that does not ferment sorbitol but produces an active GUD. This strain, designated G5101, was isolated in April 1994 from a 20-year-old female student at the University of Washington, who had bloody diarrhea. She was not hospitalized. The source of the O157:H7 isolate that caused the gastrointestinal illness was not determined.

Serotyping with anti-O157 and anti-H7 sera identified strain G5101 as O157:H7. Furthermore, colony hybridization analysis using a serotype-specific DNA probe (PF-27) also confirmed G5101 as O157:H7 serotype (Fig. 1). PF-27 is an oligonucleotide probe, specific for a unique base substitution in the allele of the *uidA* gene in O157:H7 isolates (2). However, a previous study showed that the probe also detected phenotypic variants of O157 serotype that were nonmotile, fermented sorbitol, and like our G5101 strain, exhibited GUD activity (2). These atypical pathogenic O157 strains were isolated from HUS patients in Germany and obtained from H. Karch (5).

PCR analysis of strain G5101 showed that it carried virulence genes typically present in O157:H7 strains. Amplification of the SLT genes using primers and conditions described previously yielded amplicons with predicted sizes for SLT-I and SLT-II of 475 and 863 bp, respectively (8). The production of both toxins was confirmed with a commercially available enzyme immunoassay (Meridian Diagnostics, Cincinnati, Ohio). Amplification of the *eaeA* gene using primers from the central region of the gene, which is conserved between EHEC and enteropathogenic strains of *E. coli* (forward primer from nucleotides 2241 to 2263 and reverse primer from nucleotides 3350 to 3328 of the sequence reported by Jerse et al. [6]), and conditions described above for the SLT genes yielded an amplicon of the predicted size of 1,110 bp.

Biochemical characterization showed that, in addition to having an atypical GUD phenotype, G5101 was also urease positive in 48 h. Although *E. coli* strains are usually negative

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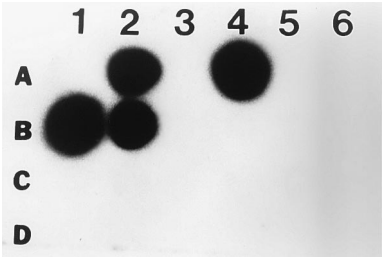

FIG. 1. Autoradiogram of colony hybridization of *E. coli* O157:H7-specific PF-27 probe to *E. coli* and other enteric bacteria. The probe assay was done as previously described (2). The serotype of each isolate is shown in parenthesis. The positive controls are isolates EC177 (O157:H7) (A2), ATCC 35150 (O157:H7) (B1), and ATCC 43888 (O157:H7) (B2). The negative controls are isolates FDA207 (*Escherichia hermannii*) (B4) and FDA400 (H10407) (B5). The samples are isolates G5101 (O157:H7) (A4), ATCC 29026 (*S. dysenteriae*) (C1), ATCC 35401 (O78:H11) (C2), ATCC 43886 (O25:K98:NM) (C3), ATCC 43887 (O111) (C4), ATCC 43893 (O124:NM) (C5), and ATCC 43896 (O78:K80:H12) (C6). All other positions on the colony filter are blanks.

for urease (1), 24 cases of hemorrhagic colitis were caused by SLT-producing, urease-positive *E. coli* O157 in Canada through 1991 (9).

There is increasing evidence suggesting that phenotypic variations exist among the isolates within *E. coli* O157:H7. In Germany, Gunzer et al. (5) found that of 44 SLT-II-producing *E. coli* O157 strains isolated from patients with diarrhea or HUS, 17 fermented sorbitol and were GUD positive. Phenotypic variants of *E. coli* O157 have also been isolated in other parts of central Europe (3). Strain G5101 is the first phenotypic variant of the O157:H7 serotype isolated in the United States. Clinical and food microbiologists should be aware of the emergence of these phenotypic variants and be cognizant that these strains may not be identified by routine culture methods or by biochemical tests used to characterize serotype O157:H7.

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