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Characterization of a *gne*::IS*629* O Rough:H7 *Escherichia coli* Strain from a Hemorrhagic Colitis Patient▽

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Shiga-toxigenic *Escherichia coli* strains that are O rough:H7 due to *gne*::IS*629* were thought to be rare and to have unknown pathogenic potential. Recently, an O rough:H7 strain caused by *gne*::IS*629* was isolated from a hemorrhagic colitis patient, suggesting that these strains are pathogenic and may not be as rare as anticipated.

Serotype O157:H7 is the prototypic Shiga-toxigenic *Escherichia coli* (STEC) strain that causes food-borne infections worldwide, and it is identified by the presence of the somatic (O)157 and the flagellar (H)7 antigens. Previously, we characterized MA6, an O rough:H7 strain that did not express the O157 antigen (5), and found that it was an O157:H7 strain that belonged in the most common O157:H7 clonal type. Despite the absence of O157 antigen expression, MA6 had all of the genes and operons essential for O157 antigen synthesis, but it exhibited the O rough phenotype due to an IS*629* insertion in the *gne* gene (*gne*::IS*629*) (8), which encodes an epimerase enzyme essential for the synthesis of an oligosaccharide subunit in the O antigen. The IS*629* element is commonly present (and in multiple copies) in enteric bacteria, including O157:H7 strains, and has been reported to cause gene disruptions (6). However, MA6-like O157:H7 strains that are O rough due to *gne*::IS*629* were thought to be rare, as MA6 was the only strain isolated thus far. Moreover, since MA6 was isolated only from a beef sample in Malaysia and was not implicated in illness, the pathogenic potential of this strain was also uncertain. A study in Germany characterized patient isolates of STEC over a 3-year period and found a few strains with the O rough:H7 phenotype (1). One of these, CB7326, was isolated from a hemorrhagic colitis patient and found to carry Shiga toxin 1 (stx_1), Shiga toxin 2 (stx_2), and γ-intimin (γ-*eae*) genes, which are common in O157:H7 strains, suggesting that, like MA6, CB7326 may be an O rough variant of O157:H7. In this study, we characterized strain CB7326 to determine the cause of its O rough phenotype and compared it to MA6 to determine whether these are analogous or related strains.

Strain CB7326 was plated on sorbitol MacConkey agar with ColiComplete (BioControl, Bellevue, WA), and it was determined that the strain did not ferment sorbitol or exhibit ß-glucuronidase activity. Serological analysis by latex agglutination (RIM O157:H7; Remel, Lenexa, KS) confirmed the presence of the H7 antigen but not the O157 antigen. Despite the absence of serological reactivity, however, PCR analysis (7) for the *wzx* and *fliC* genes, which encode the O157 and H7 antigens, respectively, confirmed that CB7326 carried genetic sequences for both antigens. By use of a multiplex PCR (4), CB7326 was found to carry typical enterohemorrhagic *E. coli* (EHEC) virulence markers, including stx_1, stx_2, *ehxA* (enterohemolysin), the γ-*eae* allele, and the +93 *uidA* (ß-glucuronidase) single nucleotide polymorphism, which is unique to O157:H7. Except for the absence of the O157 antigen, these traits are consistent with those of O157:H7 (strain EDL931). Strain CB7326 had traits identical to those of strain MA6, except that MA6 did not carry stx_1 (Table 1).

To determine whether the cause of the O rough phenotype in CB7326 was also due to *gne*::IS*629*, the *gne* gene of CB7326 was amplified by PCR using primers and parameters described previously (8). Analogous to the findings with MA6, CB7326 yielded a larger amplicon (~2,700 bp in size) than did O157:H7 (strain EDL931) (1,400-bp product) (data not shown). Sequence and BLAST analyses of the 2,700-bp amplicon confirmed that the *gne* gene of CB7326 also had the IS*629* element; however, unlike in MA6, where *gne*::IS*629* was found at position +385, *gne*::IS*629* in CB7326 was located at position +711. *trans*-Complementation of CB7326 with the pGNE construct, which carried a wild-type *gne* insert (8), restored O157 antigen expression to that for CB7326 as determined by a serological assay (data not shown). These results confirm that, as for MA6, the O rough phenotype of CB7326 was due to *gne*::IS*629*.

Multilocus sequence typing analysis using 7 specific housekeeping genes (http://www.shigatox.net/ecmlst/cgi-bin/index) confirmed that CB7326 was of sequence type 66 (ST-66) and, therefore, belongs in the most common clonal type of O157:H7 strains. However, a pulsed-field gel electrophoresis (PFGE) comparison of XbaI-digested genomic DNA showed that MA6 and CB7326 shared little homology with EDL931 or with each other and, therefore, are not analogous strains (data not shown). Still, the isolation of another O rough:H7 strain due to *gne*::IS*629* suggests that these strains may not be as rare as previously anticipated (8). The insertion of IS*629* at two different *gne* locations may have been coincidental, or there may be multiple IS*629* insertion sites within *gne*. If so, however, it

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TABLE 1. Comparison of traits and markers among MA6, CB7326, and O157:H7 strains

Trait or marker[a]	Result for strain:		
	MA6	CB7326	O157:H7[b]
SOR	−	−	−
GUD	−	−	−
O157	−	−	+
wzx (O157)	+	+	+
H7	+	+	+
fliC (H7)	+	+	+
stx_1	−	+	+
stx_2	+	+	+
γ-*eae*	+	+	+
uidA	+	+	+
ehxA	+	+	+
MLST	ST-66	ST-66	ST-66
gne::IS*629*	+385	+711	−

[a] SOR, sorbitol fermentation; GUD, ß-glucuronidase activity; O157 and H7, O157 and H7 antigens, tested by latex agglutination; *wzx* (O157), *wzx*, encoding the O157 antigen; *fliC* (H7), *fliC*, encoding the H7 antigen; stx_1 and stx_2, Shiga toxin 1 and 2 genes, respectively; γ-*eae*, γ-intimin allele; *uidA*, +93 *uidA* single nucleotide polymorphism; *ehxA*, enterohemolysin; MLST, multilocus sequence typing; *gne*::IS*629*, insertion location.

[b] Strain EDL931.

would seem that such O rough:H7 strains would have been encountered more often. The IS*629* insertion site sequence is unknown, so at this time, the existence of an IS*629* hot spot(s) within *gne* remains inconclusive. Lastly, O rough strains of other STEC serotypes have been implicated in infections and cases of hemolytic-uremic syndrome (2, 3), but the pathogenic potential of MA6 is uncertain, as it has been isolated only from foods. Although MA6 and CB7326 are not analogous strains, they are similar in that both are O rough due to *gne*::IS*629*, and the fact that CB7326 was isolated from a hemorrhagic colitis patient suggests that MA6 and other similar O rough:H7 strains, if found, may also be pathogenic to humans.

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