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Characterization of Nonmotile Variants of *Escherichia coli* O157 and Other Serotypes by Using an Antiflagellin Monoclonal Antibody

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An antiflagellin monoclonal antibody (15D8) was used to detect the presence of flagella in nonmotile variants of several pathogenic *Escherichia coli* serotypes. Of the 48 isolates examined, 15 reacted with monoclonal antibody 15D8 and were culturally confirmed to be motile. Of the 38 clinical strains designated O157:NM or O157:H$^-$, 7 were antibody reactive and motile and agglutinated with anti-H7 sera.

Escherichia coli serotype O157:H7 has emerged as a predominant cause of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) in humans (8). This pathogen is characterized serologically by the presence of the somatic O157 and the flagellar H7 antigens. Recently, however, nonmotile variants of the O157 serotype have been isolated more frequently worldwide, and some have been implicated in illnesses. These variants, designated NM or H$^-$ (2), can produce Shiga-like toxins (SLT) and have been isolated from fecal samples from HC and HUS patients in Germany (3) and the United Kingdom (14). In Central Europe, the isolation rate of nonmotile variants has sometimes surpassed that of O157:H7 (2, 3). Nonmotile variants of other pathogenic *E. coli* serotypes also exist, including, for example, O111:NM from recent outbreaks of HUS in Italy and Australia (4). To determine if the absence of motility in these variants was due to the absence of flagella, a monoclonal antibody (MAb), 15D8, which recognizes a common epitope on the enteric flagellin (7) was used in this study to examine nonmotile variants of O157 and other *E. coli* serotypes by Western blotting (immunoblotting).

A subset of 48 nonmotile variants examined included a few reference strains (American Type Culture Collection, Rockville, Md.) and isolates from food-borne outbreaks (U.S. Department of Agriculture and State Health Departments of Washington and Oregon), but most were clinical strains isolated from diarrhea, HC, and HUS patients from the United States, Germany, and Japan. Generally, isolates are classified as nonmotile after repeated culturing and microscopic examinations fail to show motility. Because of a lack of standard nomenclature, nonmotile variants are designated either NM or H$^-$. The original designations are used in this paper. All nonmotile isolates were examined on Motility Test Medium (Becton Dickinson Microbiology Systems, Cockeysville, Md.) to confirm the absence of motility, and NM variants of the O157 serotype were verified serologically with anti-O157 sera (REMEL, Lenexa, Kans.). The toxigenic potentials of these isolates were also determined by PCR using primers specific for the SLT-I and -II genes (5).

A previous analysis of 280 bacterial isolates showed that MAb 15D8 is specific for enteric flagella (7). However, to ensure that the antibody recognizes various H types, seven *E. coli* strains with different H serotypes were examined by Western blotting. Cell proteins fractionated by discontinuous (3% stacking–10% separating) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were transferred to nitrocellulose paper and probed with a 1:5,000 dilution of an ascites preparation of MAb 15D8 (7). Antigen-antibody complexes were detected with a 1:2,500 dilution of alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) and a precipitable substrate consisting of 0.48 mM nitroblue tetrazolium and 0.56 mM bromo-chloroindolyl phosphate (Sigma, St. Louis, Mo.) in 0.1 M Tris–0.1 M NaCl–5 mM $MgCl_2$, pH 9.5. The sizes of antibody-reactive proteins were estimated with prestained low-M_r protein markers. All *E. coli* isolates of various H serotypes produced single antibody-reactive proteins whose M_rs ranged from 52,000 to 69,000 (Fig. 1). *E. coli* flagellins have been reported to vary in size; their M_rs can range from 36,000 to 69,000 (7, 11, 18). The M_r of H7 flagellin, deduced from the DNA sequence, is 59,000 (13); however, SDS-PAGE studies estimate it to be 61,000 to 67,000 (11, 15, 18). From our analysis, the M_rs of H7 flagellins of the O157:H7 and O55:H7 serotypes were found to be identical and were estimated to be 69,000 (Fig. 1, lanes 1 and 7).

Having established the specificity of MAb 15D8 for various H types, 48 nonmotile variants of several *E. coli* serotypes were examined for the presence of flagella. Of these, 13 were found to produce an antibody-reactive protein. The presence of flagella, however, is not indicative that these strains are motile. Relaxed selection could have caused the loss of motility functions, these bacteria could carry cryptic flagellum genes (1, 17), or they could be defective in other mechanisms of flagellum assembly or motility.

To determine if these flagellin-producing isolates were actually motile, wet mounts of cultures were examined by phase-contrast microscopy. Additionally, because motility can sometimes be induced in nonmotile isolates (7), selected nonmotile variants that were nonreactive or weakly reactive with the antibody were subjected to motility induction. Cells were grown in M broth (Difco, Detroit, Mich.) at 30°C to stimulate flagellin production and were then point inoculated into Motility Test Medium. After overnight growth at 30°C, the cul-

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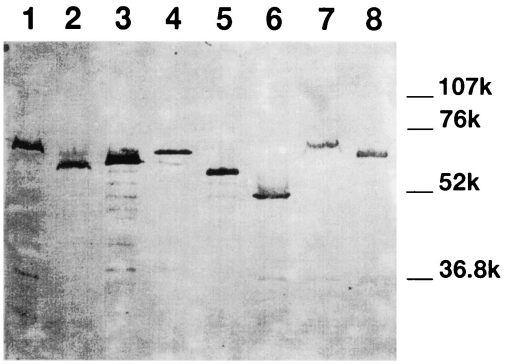

FIG. 1. Immunoblot of various *E. coli* H serotypes, using antiflagellin MAb 15D8. The serotypes shown are O157:H7 (lane 1), O157:H16 (lane 2), O157:H45 (lane 3), O157:H12 (lane 4), O157:H3 (lane 5), O157:H38 (lane 6), O55:H7 (lane 7), and O153:H2 (lane 8). The sizes (in thousands [k]) of prestained low-M_r markers (Bio-Rad, Richmond, Calif.) are shown on the right.

tures were examined for motile flares radiating from the point of inoculation. This procedure was repeated three times for each strain, and then the cultures were reexamined by microscopy and immunoblotting with MAb 15D8. All isolates were also analyzed by PCR after motility induction to confirm that they had identical SLT profiles.

Of the 10 non-O157 serotypes examined, six isolates reacted with the antibody and were confirmed to be motile by microscopy and in Motility Test Medium (Table 1). Of the four isolates that did not react with the antibody, two were unaffected by induction and remained nonmotile; however, motility was induced in two other strains (ATCC 43886 and FDA403) and they subsequently became antibody reactive on Western blots (Table 1 and Fig. 2).

In the O157 serogroup, 7 of the 38 NM and H^- strains examined reacted with the antibody, and 4 of these were strongly reactive and confirmed by microscopic examination to be motile (Table 1). The other three strains showed weak antibody reactivity, and microscopic analysis verified that only a partial population in these cultures was motile. When these three isolates were subjected to induction, however, motility was enhanced and all three strains became strongly reactive with MAb 15D8 (Table 1 and Fig. 2). Immunoblots of these seven motile or motility-enhanced variants of O157:NM serotype produced a single antibody-reactive protein that was identical in size to the H7 flagellin. Furthermore, serological analysis showed all seven isolates to agglutinate with anti-H7 sera, thus confirming that they carry the H7 antigen. Eight additional NM isolates of the O157 serotype that did not react with the antibody were also subjected to motility induction; however, none of these became motile and all remained nonreactive with MAb 15D8 (Table 1).

The presence of the H7 antigen in all motile and motility-enhanced isolates of serotype O157 suggests that a proportion

TABLE 1. Motility and MAb 15D8 reactivity of nonmotile variants of *E. coli*[a]

Strain	Serotype	Source	SLT type		Result BI[b]		Result AI[c]	
			I	II	Mot[d]	MAb 15D8 reactivity	Mot	MAb 15D8 reactivity
TB285	O26:H$^-$	Diarrhea	+	−	+	+	ND[e]	ND
TB352	O26:H$^-$	Diarrhea	+	−	+	+	ND	ND
TB226	O68:H$^-$	HC patient	+	+	−	−	−	−
43893	O124:NM	ATCC[f]	−	−	−	−	−	−
43886	O25:K98:NM	ATCC	−	−	−	−	+	+
13A71	O48:H$^-$	Meat	+	+	+	+	ND	ND
FDA321	O55:NM	Diarrhea	−	−	+	+	ND	ND
FDA403	O111:NM	Stool	+	+	−	−	+	+
FDA405	O111:NM	Bovid	+	−	+	+	ND	ND
FDA401	O125:NM	Stool	−	−	+	+	ND	ND
13180-NM	O157:NM	Meat	−	+	±	±	+[g]	+
7123	O157:NM	Meat	−	−	−	−	−	−
H0482	O157:NM	HUS patient	−	+	−	−	−	−
G5918	O157:NM	Diarrhea	+	+	+[g]	+	ND	ND
G6001	O157:NM	Diarrhea	+	+	+[g]	+	ND	ND
3295-91	O157:NM	Clinical specimen	−	+	±	±	+[g]	+
3030-92	O157:NM	Diarrhea	+	+	±	±	+[g]	+
3204-92	O157:NM	Clinical specimen	−	+	−	−	−	−
E32511	O157:NM	HUS patient	−	+	−	−	−	−
13B42	O157:NM	HC patient	−	+	+[g]	+	ND	ND
13B47	O157:NM	HC patient	+	+	−	−	−	−
TT7	O157:H$^-$	HC patient	+	−	−	−	−	−
TT13	O157:H$^-$	HC patient	+	+	+[g]	+	ND	ND
493bi	O157:H$^-$	HUS patient	−	+	−	−	−	−
5412/1	O157:H$^-$	HUS patient	−	+	−	−	−	−

[a] Only 15 of the 38 O157:NM or O157:H$^-$ isolates were subjected to induction. +, −, and ±, presence, absence, and partial presence of toxin, motility, or weak antibody reactivity, respectively.
[b] BI, before induction.
[c] AI, after induction.
[d] Mot, motility, visualized by phase-contrast microscopy.
[e] ND, not done. (Motile isolates were not subjected to induction.)
[f] ATCC, American Type Culture Collection.
[g] Agglutinated with anti-H7 latex.

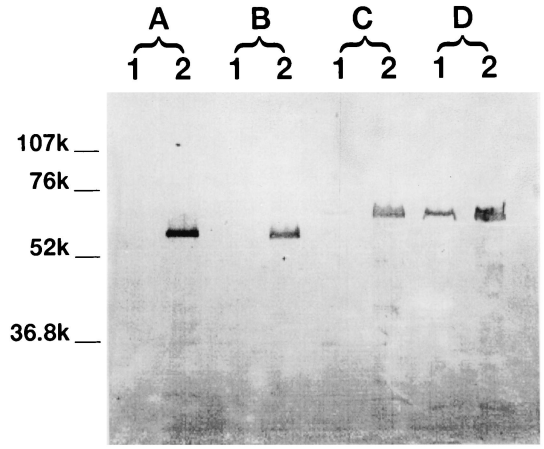

FIG. 2. Immunoblot of nonmotile strains (lanes 1) and their motility-induced or -enhanced variants (lanes 2), using antiflagellin MAb 15D8. The strains shown (with the serotypes indicated in parentheses) are as follows: A, ATCC 43886 (O25:K98:NM); B, FDA403 (O111:NM); C, 3030-92 (O157:NM); and D, G5918 (O157:NM). The protein concentrations loaded for each strain and its motile variant were standardized on the basis of the A_{280}. The sizes (in thousands [k]) of prestained low-M_r markers are shown on the left.

of the strains designated NM and H^- are mistyped and could be isolates of O157:H7 that are nonmotile because of a variety of factors. This assumption is supported by our finding that of the 38 nonmotile O157 variants which were obtained from various sources worldwide, 35 carried SLT gene sequences, reacted with an O157:H7-specific DNA probe (6), and exhibited sorbitol and β-glucuronidase phenotypes identical to those of O157:H7. Furthermore, these nonmotile variants also had electrophoretic type profiles identical to that of O157:H7 on multilocus enzyme electrophoresis analysis (data not shown) and, hence, appeared to be in the O157:H7 clonal group.

Nonmotile strains are usually induced extensively for motility before being designated NM or H^-. It was surprising, therefore, that 15 of the 48 NM isolates examined, including one reference strain, were actually motile. The mechanism of motility in *E. coli* is complex and under intricate regulatory control. It is also affected by environmental factors, as flagellum biosynthesis can be repressed in medium containing glucose or in the presence of various catabolites (12). It is possible, therefore, that some of these NM variants were under catabolite repression; however, motility was restored after induction or derepression.

The absence of motility could also be attributed to genetic factors. Twenty-two NM and H^- variants of serotype O157 examined in this study were tested by PCR (13) and were found to carry the entire *fliC* gene (data not shown); hence, they had the structural genes that code for flagella. This, however, does not rule out the presence of deletions and mutations that could cause the absence of flagellum synthesis. Also, the O157 serogroup is large and genetically diverse, so that not all NM variants are closely related (19). For example, pulsed-field gel electrophoresis studies showed that some phenotypic variants of serotype O157:H^- from Germany had distinct genetic profiles and might represent a clonal group different than O157:H7 (10). Two of these pathogenic, SLT-II-producing phenotypic and motility variants (493bi and 5412/1) were examined in this study; neither strain reacted with the antiflagellin antibody, and in neither strain was motility induced (Table 1). Hence, in some cases the absence of motility could be a clonal characteristic of some serotypes.

Finally, there is some evidence to suggest that the absence of motility could be correlated with bacterial pathogenesis (16). Human pathogens such as *Bordetella* (1) and *Shigella* (17) spp. are nonmotile, yet both carry intact but cryptic flagellin genes. Also, in *Yersinia enterocolitica* a temperature shift to 37°C causes a coordinate repression of motility with the induction of virulence genes (9), perhaps to switch the cell's energy expenditure from motility to virulence. The frequent isolation of O157:NM and O157:H^- variants from clinical infections, coupled with our findings that most of these variants are in the H7 clonal group, makes it tempting to speculate that perhaps similar correlations between motility and virulence in the O157:H7 serotype exist.

In conclusion, 8 of the 10 NM variants of several *E. coli* serotypes other than O157, including one reference strain, were found to be motile. Of the 38 clinical or food isolates designated O157:NM or O157:H^-, 35 were found to be in the same clonal group as O157:H7, and of these, the seven isolates that reacted with the antiflagellin antibody were confirmed to be motile and agglutinated with anti-H7 sera.

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