

Comparison and Genomic Sizing of *Escherichia coli* O157:H7 Isolates by Pulsed-Field Gel Electrophoresis

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Genomic DNAs of *Escherichia coli* O157:H7 strains isolated from patients and food samples were analyzed by pulsed-field gel electrophoresis. The rare-cutting endonucleases *Sfi*I and *Xba*I generated 6 and 10 distinct genomic profiles, respectively, for the 22 strains analyzed, indicating that this technique may find application for epidemiologic studies. Summation of *Xba*I fragments from five *E. coli* O157:H7 strains estimated the genomic length at ca. 4.7 Mb.

Escherichia coli O157:H7 causes a spectrum of illnesses ranging from hemorrhagic colitis to hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura (8). Since first being recognized as a food-borne pathogen in 1982, this specific serotype of *E. coli* has been responsible for at least 14 deaths in food-related outbreaks in the United States (8). Although ground beef is often cited as the primary vehicle of transmission, turkey roll, raw milk, and person-to-person transmission have also been implicated (6). Because of its presence in raw foods and the potential severity of illness and attendant costs, the development of better methods to monitor this bacterium remains an active area of research. In this regard, epidemiological investigations of food-borne illness involving *E. coli* have benefited greatly from the advent of molecular typing strategies (for examples, see references 1, 8, 10, 14, 15, and 19). As described herein, the pulsed-field gel electrophoresis (PFGE) technique of contour-clamped homogeneous electric field (CHEF) electrophoresis was used to estimate genome size and decipher molecular differences among otherwise phenotypically similar *E. coli* O157:H7 isolates.

Preparation, digestion, and fractionation of high-molecular-weight genomic DNA in agarose plugs. The O157 serotype of isolates was verified by an *E. coli* O157 coagglutination test (Oxoid, Basingstoke, England). Prior to CHEF, *E. coli* cultures were transferred twice in Luria broth (13) for 18 h at 37°C with shaking (200 rpm). Then the cells were harvested, washed, suspended in TE buffer (13), and adjusted to an appropriate density for preparation of agarose plugs as previously described (9). Agarose plugs containing cells of *E. coli* were processed as described previously (12), and genomic DNA within agarose plugs was then digested with *Sfi*I (New England BioLabs, Beverly, Mass.) or *Xba*I (Promega Corp., Madison, Wis.) as recommended by the manufacturer. High-molecular-weight restriction fragments were resolved by using a CHEF (CHEF-DR II; Bio-Rad Laboratories, Richmond, Calif.) PFGE system and electrophoresis-grade agarose (1.0%; GIBCO-Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, Md.) as described previously (12). The electrophoretic regimen of 200 V for ca. 21 h at 15°C and the following switch times was used to fractionate and visualize restriction fragments: (i) <200 kb, ramped from 0.1 to 15 s; (ii) <350 kb, ramped from

10 to 20 s; and (iii) <700 kb, ramped from 15 to 40 s or from 1 to 35 s. The lambda ladder (48.5-kb increments) and *Saccharomyces cerevisiae* chromosome (245- to 2,200-kb) standards (Bio-Rad) were used as molecular size markers.

Genome length. The sizes and numbers of restriction fragments generated by digestion of intact genomic DNA from five *E. coli* O157:H7 strains with *Xba*I are listed in Table 1. Summation of fragment lengths approximated the genome size between 3.6 and 5.6 Mb (average, 4.7 Mb). *Sfi*I digestion of intact genomic DNA from the same five strains followed by summation of fragment lengths estimated the genome size to be between 3.3 and 4.0 Mb (average, 3.5 Mb [data not shown]). Possible sources of error in calculating the genome size of *E. coli* O157:H7 isolates by using either enzyme include, but are not limited to, the presence or absence of plasmids and prophages, the chromosomal location of prophages, and the contribution of small (<20-kb) fragments. However, it is not uncommon to encounter variability in genome length among related strains (4, 5, 18, 23). Regarding the size discrepancy between the summation of *Xba*I and *Sfi*I fragments, Perkins et al. (17) also experienced some difficulty in establishing an *Sfi*I cleavage map for *E. coli* MG1655: (i) some *Sfi* sites overlapped with the recognition site for the *dcm* methylase, resulting in inefficient cutting at some *Sfi* sites, and/or (ii) digestion with *Sfi*I produced several comigrating (i.e., doublet) fragments, notably doublets of about 370, 300, 160, and 79 kb, that required additional strategies to identify. Similar mechanisms may be operating in *E. coli* O157:H7 and contributing to spurious size estimates; therefore, more exhaustive studies may be necessary to estimate the genome size of serotype O157:H7 strains following digestion with *Sfi*I.

Other investigators (3) have used PFGE to compare genomic DNAs from *E. coli* O157:H7, but there have been no reports estimating genome length using this technique. Our results estimating genome size for *E. coli* O157:H7 with *Xba*I (ca. 4.7 Mb) are in agreement with the genome size of other *E. coli* strains. For example, summation of the total fragment sizes from digestion of *E. coli* K-12 strain MG1655 with *Avr*II and with *Sfi*I predicts chromosome lengths of 4,835 and 4,557 kb, respectively (5, 17). In another study with the enzymes *Not*I and *Sfi*I, the size of the *E. coli* K-12 genome was estimated at 4,696 and 4,711 kb, respectively (21). The use of specific probes, additional enzymes, and/or two-dimensional electrophoresis concomitant with comparisons with more detailed physical and genetic maps and

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TABLE 1. CHEF analysis of *E. coli* O157:H7 genomic DNA digested with *Xba*I^a

Fragment	Fragment size (kb) for strain:				
	ATCC 43889	ATCC 43894	ATCC 43895	505B	RM5
1	19.4	26.7	26.7	19.4	29.1
2	31.5	38.8	38.8	34.0	42.1
3	38.8	65.5 ± 21.8	65.5 ± 21.8	40.01	55.8
4	53.4	75.2 ± 26.7	75.8 ± 23.7	50.9	67.9
5	58.2	83.1 ± 29.7	83.1 ± 29.7	58.2	75.2
6	60.6	100.6 ± 18.2	100.6 ± 18.2	72.8	96.6 ± 8.1
7	77.6	114.0 ± 31.5	113.4 ± 32.1	75.2	111.6 ± 4.9
8	93.4	130.4 ± 23.7	130.4 ± 23.7	89.7	126.1 ± 0
9	128.5 ± 17.0	160.1 ± 34.0	158.9 ± 35.2	111.6 ± 4.9	149.1 ± 3.6
10	133.4 ± 17.0	174.6 ± 19.4	173.4 ± 35.2	126.1 ± 2.4	166.1 ± 8.5
11	145.2 ± 19.1	188.0 ± 18.2	187.6 ± 36.4	145.1 ± 4.4	175.8 ± 10.9
12	152.8 ± 17.0	220.8 ± 14.7	219.6 ± 35.0	163.7 ± 6.0	209.8 ± 8.5
13	163.0 ± 17.5	235.4 ± 14.7	234.2 ± 32.6	174.6 ± 9.7	225.5 ± 2.4
14	179.5 ± 19.4	245.7 ± 15.3	243.9 ± 32.6	210.5 ± 9.2	245.0 ± 7.3
15	191.0 ± 16.4	257.8 ± 15.3	255.4 ± 30.8	235.4 ± 7.4	265.5 ± 3.6
16	222.6 ± 16.5	280.4 ± 28.2	278.6 ± 34.8	251.6 ± 6.7	278.9 ± 2.4
17	247.6 ± 12.4	314.9 ± 36.1	313.1 ± 29.4	263.7 ± 4.2	291.0
18	262.2 ± 19.7	411.0 ± 37.6	410.2 ± 43.8	278.3 ± 6.7	303.1
19	284.6 ± 20.3	490.9 ± 76.7	486.2 ± 108.5	310.6 ± 14.7	310.4
20	306.5 ± 30.0	519.3 ± 78.0	513.7 ± 110.1	335.7 ± 15.6	334.7
21	365.3 ± 74.3	688.1 ± 115.2	668.1 ± 135.2	382.5 ± 38.2	
22	536.5 ± 72.8			404.4 ± 45.5	
23	553.5 ± 70.3			480.3 ± 77.8	
24	608.0 ± 95.8			633.5	
25	638.4				
Total	5,551.5	4,821.3	4,776.3	4,947.8	3,559.3

^a The genomic DNA was estimated to be 4,731 kb (mean of the five totals in the table). Individual and total fragment sizes were calculated as an average of at least two different gels for fragments greater than 100 kb.

libraries of *E. coli* strains will augment our efforts to ascertain the precise size and orientation of *E. coli* O157:H7 macrorestriction fragments (i.e., to establish a more sophisticated physical and genetic map). The further exploitation of PFGE-CHEF technology should also prove invaluable for molecular tracking of *E. coli* O157:H7 strains for epidemiologic studies.

Comparison of *E. coli* O157:H7 strains by PFGE. In preliminary experiments, the enzymes *Not*I, *Sse* 83871, *Avr*II, *Srf*I, and *Sgr*AI were evaluated. These enzymes were not as useful as *Xba*I or *Sfi*I because of an overabundance of bands and/or the inability to resolve the resulting fragments under the electrophoretic conditions used. Therefore, *Xba*I and *Sfi*I were used to assess the genetic relatedness among strains by comparison of restriction endonuclease digestion profiles (REDP). For 22 isolates screened, CHEF analyses revealed 10 REDP following digestion with *Xba*I (Table 2) and 6 REDP following digestion with *Sfi*I (Table 3). The rare-cutting endonuclease *Xba*I separated strains into more REDP than did *Sfi*I; therefore, *Xba*I is potentially more useful for detecting variability (i.e., REDP) among strains. More specifically, strains CL-8, HA1, ATCC 43895, and ATCC 43894 of *Sfi*I REDP I each displayed a unique REDP when digested with *Xba*I. As another example, although strains 264 and RM14 made up a single *Xba*I REDP, both strains were part of a larger group of strains in *Sfi*I REDP II. In contrast, strain 204-P occupied a unique *Sfi*I REDP but shared an *Xba*I REDP with strain 260. These results underscore the importance of using more than one enzyme for molecular typing via PFGE to discern subtle differences among strains. Additionally, these data indicate that differences in REDP exist among O157:H7 isolates and that

TABLE 2. REDP of *E. coli* O157:H7 genomic DNA obtained with *Xba*I

REDP	Strain	Source
I	262	Hamburger
	RM10	Raw milk
	RM11	Raw milk
	RM12	Raw milk
	RM5	Raw milk
	RM7	Raw milk
II	RM9	Raw milk
	RM13	Raw milk
	505B	Beef roast
	RM2	Raw milk
	RM3	Raw milk
III	260	Patient
	204-P	Meat
IV	264	
	RM14	Raw milk
V	CL-8	Patient
VI	87-3	Patient
VII	HA1	Rough derivative of 932
VIII	ATCC 43895	Ground beef
IX	ATCC 43894	Stool
X	ATCC 43889	Stool

TABLE 3. REDP of *E. coli* O157:H7 genomic DNA obtained with *Sfi*I

REDP	Strain	Source
I	262	Hamburger
	RM10	Raw milk
	RM11	Raw milk
	RM12	Raw milk
	CL-8	Patient
	HA1	Rough derivative of 932
	RM5	Raw milk
	RM7	Raw milk
	ATCC 43895	Ground beef
	ATCC 43894	Stool
II	264	
	RM9	Raw milk
	RM13	Raw milk
	RM14	Raw milk
	505B	Beef roast
	RM2	Raw milk
	RM3	Raw milk
III	260	Patient
IV	204-P	Meat
V	87-3	Patient
VI	ATCC 43889	Stool

CHEF is more discriminatory than serology for typing *E. coli* O157:H7 strains.

The genomic fingerprints obtained following digestion of nine representative *E. coli* O157:H7 strains and four other *E. coli* strains with *Xba*I are shown in Fig. 1. As also reported

by Böhm and Karch (3), some bands were common among all O157:H7 isolates examined; however, for some strains differences were observed in the migration of fragments of about 580, 340, and 97 kb. Comparison of genomic fingerprints generated with *Xba*I also revealed differences between phenotypically indistinguishable paired strains of *E. coli* O157:H7 isolated from patients and food products (compare the most slowly migrating bands in lanes J and K in Fig. 1) and among strains from the same food source (e.g., milk isolates; compare lanes A and C). CHEF analyses also revealed differences between O157:H7 strains and *E. coli* strains of other serotypes (compare lanes H, L, and M with the other lanes), including two enterohemorrhagic serotype O26:H11 isolates (lanes L and M).

There have been few if any studies comparing the stabilities of REDP in response to food components, host passage, environmental stimuli, and/or repeated storage and transfer of strains. Kazmi et al. (11) reported that virulence of *Campylobacter jejuni* was enhanced by animal passage but did not indicate whether this resulted from a genetic change(s). In contrast, in vivo passage of *E. coli* did not alter the profile of restriction fragments (1). Likewise, there have been at least two reports indicating that genomic fingerprints of *Listeria monocytogenes* were stable after animal passage (2, 22). However, both studies used restriction enzyme analysis, and it is possible that subtle differences in the banding pattern were not noticed, particularly in lower-molecular-weight fragments, because of the number of bands generated. As another example, polymorphisms were detected in ribosomal sequences of *Candida albicans* following laboratory passage (20). Thus, it seems prudent to determine whether various stimuli affect the genomic profile and, as such, have an impact on the virulence potential of strains.

Several methods have been used with varying success to type *E. coli* strains, including serotype O157:H7 strains (for example, see references 1, 3, 7, 10, 16, 19, and 24). Of these



FIG. 1. Comparison of *Xba*I digests of *E. coli* genomic DNA by CHEF. *E. coli* O157:H7 isolates are in lanes A (RM11), C (RM9), D (264), E (204-P), F (87-3), G (CL-8), I (ATCC 43889), J (ATCC 43894), and K (ATCC 43895). Non-O157:H7 *E. coli* isolates are in lanes B (rough derivative of O157; HA1), H (K-12 strain MG1655), L (O26:H11; 89-326), and M (O26:H11; 88-353). The genomic fingerprints shown are identical to REDP of each strain obtained in at least three replicate gels.

methods, PFGE-CHEF is arguably the most discriminating method for typing *E. coli* strains. Arbeit et al. (1), using PFGE, obtained different restriction patterns for *E. coli* isolates from different patients even though the serotypes, antibiotic sensitivities, and electrophoretic enzyme types were identical. Likewise, when PFGE was used to separate genomic DNA and was followed by Southern hybridization with virulence-associated gene probes, *E. coli* strains within a serotype exhibited different restriction patterns (15). As another example, Gordillo et al. (7) used PFGE to distinguish between enteroinvasive *E. coli* strains of the same serogroup and phenotype and to establish genetic relatedness among serotype O143 strains. In addition, *E. coli* O157:H7 strains from various geographic locations displayed differences, albeit minor ones, in restriction patterns following digestion with *Xba*I and resolution by PFGE (3). We evaluated PFGE-CHEF as an epidemiologic tool to differentiate *E. coli* O157:H7 strains isolated from clinical and food samples. Our results corroborate those of other studies and substantiate the claim that PFGE-CHEF is a highly discriminatory typing technique that can be used to identify genomic differences among related strains. Studies are under way to evaluate the contribution, if any, of resident phage and plasmids to the genomic fingerprint of *E. coli* O157:H7 strains.

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