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Measurements of Fitness and Competition in Commensal *Escherichia coli* and *E. coli* O157:H7 Strains†

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Although the main reservoirs for pathogenic *Escherichia coli* O157:H7 are cattle and the cattle environment, factors that affect its tenure in the bovine host and its survival outside humans and cattle have not been well studied. It is also not understood what physiological properties, if any, distinguish these pathogens from commensal counterparts that live as normal members of the human and bovine gastrointestinal tracts. To address these questions, individual and competitive fitness experiments, indirect antagonism assays, and antibiotic resistance and carbon utilization analyses were conducted using a strain set consisting of 122 commensal and pathogenic strains. The individual fitness experiments, under four different environments (rich medium, aerobic and anaerobic; rumen medium, anaerobic; and a minimal medium, aerobic) revealed no differences in growth rates between commensal *E. coli* and *E. coli* O157:H7 strains. Indirect antagonism assays revealed that *E. coli* O157:H7 strains more frequently produced inhibitory substances than commensal strains did, under the conditions tested, although both groups displayed moderate sensitivity. Only minor differences were noted in the antibiotic resistance patterns of the two groups. In contrast, several differences between commensal and O157:H7 groups were observed based on their carbon utilization profiles. Of 95 carbon sources tested, 27 were oxidized by commensal *E. coli* strains but not by the *E. coli* O157:H7 strains. Despite the observed physiological and biochemical differences between these two groups of *E. coli* strains, however, the O157:H7 strains did not appear to possess traits that would confer advantages in the bovine or extraintestinal environment.

Free-living *Escherichia coli* bacteria are generally found in two environments (51). Their primary habitat is the intestines of warm-blooded animals. Their secondary habitat is water, soil, and sediment. The primary habitat of the animal host provides a warm nutrient-rich environment that supports vigorous growth and reproduction of *E. coli*. In contrast, the secondary environment is generally cool, with wide fluctuations in temperature. It is also usually nutrient limiting, such that growth rates of *E. coli* are typically low. In theory, an individual *E. coli* bacterium can be expected to spend part of its life in the primary habitat, after which it is excreted into the secondary habitat, where it and its progeny either survive until they colonize a new host or die. In order to be successful, therefore, any specific *E. coli* clone must be able to outcompete not only other microorganisms but also other *E. coli* strains in either or both of these habitats (51).

While most *E. coli* strains are harmless inhabitants of the large intestine (5), some *E. coli* strains cause disease in humans, with over 60 serotypes able to produce Shiga-like toxins (31). Strains of enterohemorrhagic *E. coli*, such as *E. coli* O157:H7, are of particular concern due to their ability to cause serious food-borne disease. Although this serotype appears to be rather widespread in the animal environment, one of the main sources of this serotype is considered to be foods of animal origin, especially beef (12, 38, 40, 41, 46, 50, 59). Cattle, like humans, serve as gastrointestinal hosts for *E. coli* (5, 6), including *E. coli* O157:H7, and cattle are generally considered to be a reservoir for *E. coli* O157:H7 (10, 25, 33, 41) Indeed, *E. coli* O157:H7 can readily be isolated from the rumen and colon of experimentally inoculated calves and is thought to colonize these sites in the adult animal as well (9, 18, 24, 27, 41). As in humans, there can be significant fluctuation in the total number of generic *E. coli* bacteria and in the number of specific *E. coli* serotypes occurring at any one time in any particular animal (11, 28, 53, 55). Thus, *E. coli* O157:H7 can be expected to compete against other commensal *E. coli* strains within the bovine intestinal tract.

Most natural environments that support the growth or survival of *E. coli* contain a mixed population comprised of numerous individual clones (5, 6, 30, 55). Success in these environments depends on at least several physiological properties. Strains having enhanced metabolic diversity and capable of utilizing a wide array of carbon sources and other nutrients would be predicted to do especially well in the secondary, nutrient-limiting habitat. Production of, and resistance to, known intraspecific antagonistic agents, such as bacteriocins, would also be useful in enabling one clone to compete successfully against other similar clones (49). Likewise, resistance to antibiotics may also confer competitive advantages on those strains. Finally, all other factors being equal, the strains that

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are able to grow most rapidly will ultimately be more successful (22, 23).

Fitness describes the overall ability of an organism to survive and reproduce and can be measured in bacteria by calculating growth rate (2, 3, 13). The environments in which a bacterium must survive have been shown to affect fitness values (45), such that the same bacterium can have different fitness values in different environments (15). Natural selection would suggest that, in order for *E. coli* O157:H7 to have emerged and be maintained in a particular niche, it must be more fit than competing commensal *E. coli* strains. The aim of this present study, therefore, was to compare general fitness properties of *E. coli* O157:H7 and commensal *E. coli* strains. Specifically, we focused on issues of nutrient utilization, competition, and fitness in environments simulating the mammalian gastrointestinal tract, the cattle rumen, and nutrient-rich and nutrient-poor extraintestinal environments. We hypothesized that *E. coli* O157:H7 would display distinctive patterns of nutrient utilization, competition, and fitness relative to those of commensal *E. coli* strains and that these differences would reflect the overall ability of O157 strains to compete against the commensal *E. coli* population.

MATERIALS AND METHODS

Bacterial strains. The strain set used in this study consisted of 81 strains of *E. coli* O157:H7, 39 commensal *E. coli* isolates, and two laboratory K-12 strains of *E. coli*, for a total of 122 strains. Of the *E. coli* O157:H7 strains, 43 were isolated from infected humans and 32 were isolated from cattle. All had been previously characterized genotypically by octamer-based genome scanning (OBGS [32]) and classified as lineage 1 (44 strains) or lineage 2 (31 strains). Another six strains were obtained from the Karch laboratory in Germany. The commensal strains included 31 *E. coli* reference strains (37) and 8 cattle strains from the culture collections at the University of Wisconsin and University of Nebraska (17). The *E. coli* O157:H7 portion of the set was designed to represent the genetic and geographic diversity of this serotype in the United States, while the commensal *E. coli* portion of the set was designed to represent the entire genetic diversity of the species (54). All cultures were maintained in 15% glycerol stocks at −80°C.

Media. All strains were routinely maintained on tryptic soy agar (TSA; Difco, Ann Arbor, Mich.) and propagated by aerobic growth at 37°C in tryptic soy broth (TSB). For both individual and competitive fitness experiments three medium conditions were used: TSB, M-9 minimal medium (4), and modified 98-5 medium (42).

Carbon source utilization. Biolog ES (Biolog, Inc., Hayward, Calif.) plates were used to detect carbon utilization on 95 substrates. Each plate included a negative water control. The Biolog microplate carbon utilization assay is based on oxidation of each carbon source and subsequent detection of the reduced (purple) form of a tetrazolium dye. Each strain was grown in TSB, harvested during log phase via centrifugation (5 min at 8,000 × *g*), washed, and resuspended to an optical density at 600 nm of 0.1 to 0.2 in 0.85% saline. Samples (150 μl) were inoculated into each well of prewarmed Biolog plates. Plates were incubated at ambient atmosphere for 24 h at 37°C. Plates were scored in a binary fashion as either positive or negative. Data were analyzed in subgroups representing pathogenic versus commensal strains, OBGS group 1 versus group 2 strains (32), human O157:H7 versus bovine O157:H7 strains, and human versus bovine commensal strains.

Bacterial competition assays. Bacterial competition assays were performed in a 122-by-122 matrix format by an agar diffusion technique. Producer and test strains were grown aerobically overnight in TSB at 37°C. Producer strain cells were centrifuged for 5 min, and the supernatants were held on ice. Test strain cells were inoculated into tempered soft TSA (20 μl of cells into 3 ml of soft agar) and overlaid onto TSA plates. Sterile 6-mm-diameter paper disks were placed on the agar, and 25 μl of each supernatant was inoculated onto each disk. Plates were incubated aerobically overnight at 37°C. Zones of clearing, if present, were recorded in millimeters. All positive tests were repeated.

Antibiotic resistance. Antibiotic susceptibility tests were performed for all 122 strains according to the National Committee for Clinical Laboratory Standards (NCCLS) standard procedures. Briefly, premeasured antimicrobial disks were applied to the surface of Mueller-Hinton agar plates, previously seeded with each strain. Included were three beta-lactam penicillins, five expanded-spectrum cephalosporins, five aminoglycosides, two sulfas, cephalothin, cefoxitin, aztreonam, trimethoprim, tetracycline, chloramphenicol, nalidixic acid, ciprofloxacin, and imipenem. Plates were inverted and incubated overnight at 37°C in an aerobic incubator. Zones of clearing were recorded in millimeters, and sensitivity or resistance was determined using standard NCCLS interpretation charts. All experiments were performed in duplicate.

Individual fitness assays. The entire strain set was randomly arrayed, in triplicate, in 96-well plates (Costar 3598). The wells were then filled with 200 μl of sterile TSB medium and incubated overnight at 37°C. Experiments were performed by addition of 1% of the overnight cultures into fresh plates filled with 200 μl of the appropriate medium. Plates were incubated under the appropriate conditions, and cell densities were determined by optical density measurement (Sunrise Microplate Reader; Phoenix Research Products, Hayward, Calif.) at a wavelength of 620 nm. Each well was read twice, and the values were averaged. Each of the three sets of plates was run in duplicate, for a total of six measurements per strain. Data were exported into an Excel file, and calculations were performed using Microsoft Excel software. Optical density measurements were converted to cell number by using a standard curve. Fitness was measured as maximum growth rate, with growth rate calculated as $[(R2 - R1)/\Delta T)]*2.303$, where R equals the log of the converted optical density reading at a wavelength of 620 nm and T equals time in hours. Reported growth rates represent the average of the maximum growth rate for all replications. Results were compared using the two-tailed Student t test. Experiments were run in four experimental conditions, simulating four different environments. These included aerobic growth in TSB, aerobic growth in M-9, anaerobic growth in TSB, and anaerobic growth in modified 98-5 rumen medium. Medium used for the anaerobic experiments was allowed to equilibrate in an anaerobic environment for 24 h before being inoculated. All anaerobic manipulations and incubations were performed in an anaerobic chamber (model 1025; Forma Scientific, Marietta, Ohio).

RESULTS

Carbon utilization. Carbon source utilization tests showed that 28 of the 95 substrates were oxidized by 100% of the *E. coli* strains tested. An additional 30 substrates were used by at least 85% of the strains within each group. Eight of these substrates were reduced by at least 85% of the strains from each subgroup, except for the K-12 strains, which means that either one or both of the K-12 strains did not oxidize these eight substrates. Ten of the 95 substrates were oxidized by less than 25% of the strains from each subgroup. The results for the remaining 27 substrates are listed in Table 1. Of particular interest were D-saccharic acid, D-sorbitol, D-serine, D-galactonic acid δ-lactone, and glycolic acid, which were oxidized by nearly 80% of the commensal strains but by less than 20% of the O157:H7 strains. Fewer differences were observed between the OBGS lineage 1 and lineage 2 strains and between cattle and human *E. coli* O157:H7 strains, with dulcitol and glycyl-L-aspartic acid showing the greatest differences.

Bacterial competition. Out of nearly 15,000 individual disk diffusion antagonism tests, 168 (1%) displayed zones of complete clearing. In 96% of these positive tests, the producer strain was an *E. coli* O157:H7 strain, compared to only 4% of the tests showing that a commensal strain was a producer. In terms of total numbers, 28 of 81 *E. coli* O157:H7 strains produced an inhibitory substance, compared to only 6 of 41 commensal strains.

Producer results can be further analyzed by subgroup. The commensal strains were active against other commensal strains and both OBGS lineage 1 and OBGS lineage 2 strains. In contrast, although the O157:H7 strains produced inhibitory substances that were active against both commensal and O157:H7 strains, there were more than three times as many

TABLE 1. Carbon source utilization[a]

Substrate	No. (%) of positive reactions						
	All (n = 122)	Commensal (n = 41)	O157				
			All O157 (n = 81)	Lineage 1 (n = 44)	Lineage 2 (n = 31)	Cattle (n = 32)	Human (n = 43)
D-Saccharic acid	48 (39)	**36 (88)**	**12 (15)**	1 (2)	5 (16)	6 (19)	0 (0)
Dulcitol	71 (58)	13 (32)	58 (72)	**41 (93)**	**11 (35)**	**15 (47)**	**37 (86)**
D-Serine	40 (33)	**33 (80)**	**7 (9)**	3 (7)	4 (13)	5 (16)	2 (5)
D-Sorbitol	55 (45)	**40 (98)**	**15 (19)**	6 (14)	8 (26)	10 (31)	4 (9)
L-Glutamic acid	70 (57)	19 (46)	51 (63)	23 (52)	22 (71)	24 (75)	21 (49)
D-Galactonic acid δ-lactone	53 (43)	**39 (95)**	**14 (17)**	7 (16)	5 (16)	9 (28)	3 (7)
D-Glucosaminic acid	19 (16)	17 (41)	2 (2)	1 (2)	0 (0)	1 (3)	0 (0)
1,2-Propanediol	22 (18)	16 (39)	6 (7)	2 (5)	0 (0)	1 (3)	1 (2)
α-Ketobutyric acid	99 (81)	29 (71)	70 (86)	42 (95)	22 (71)	23 (72)	41 (95)
Sucrose	100 (82)	22 (54)	78 (96)	44 (100)	28 (90)	30 (94)	42 (98)
L-Threonine	92 (75)	26 (63)	64 (79)	35 (80)	23 (74)	26 (81)	32 (74)
m-Tartaric acid	37 (30)	18 (44)	19 (23)	12 (27)	5 (16)	5 (16)	12 (26)
α-Hydroxyglutamic acid δ-lactone	19 (16)	12 (29)	7 (9)	3 (7)	0 (0)	2 (6)	1 (2)
α-Hydroxybutyric acid	103 (84)	30 (73)	73 (90)	43 (98)	25 (81)	26 (81)	42 (98)
Adonitol	22 (18)	15 (37)	7 (9)	0 (0)	1 (3)	1 (3)	0 (0)
Glycyl-L-aspartic acid	101 (83)	41 (100)	60 (74)	**23 (52)**	**31 (100)**	**28 (88)**	**26 (60)**
D-Threonine	16 (13)	14 (34)	2 (2)	0 (0)	1 (3)	1 (3)	0 (0)
Propionic acid	102 (84)	34 (83)	68 (84)	43 (98)	23 (74)	25 (78)	41 (95)
Mucic acid	115 (94)	34 (83)	81 (100)	44 (100)	31 (100)	32 (100)	43 (100)
Glycolic acid	37 (30)	**32 (78)**	**5 (6)**	0 (0)	0 (0)	0 (0)	0 (0)
Glyoxylic acid	57 (47)	32 (78)	25 (31)	14 (32)	6 (19)	7 (22)	13 (30)
Glycyl-L-glutamic acid	56 (46)	24 (59)	32 (40)	11 (25)	15 (48)	13 (41)	13 (30)
Glycyl-L-proline	70 (57)	36 (88)	34 (42)	12 (27)	16 (52)	14 (44)	14 (33)
p-Hydroxyphenylacetic acid	30 (25)	26 (63)	4 (5)	1 (2)	2 (6)	3 (9)	0 (0)
m-Hydroxyphenylacetic acid	30 (25)	24 (59)	6 (7)	1 (2)	4 (13)	4 (13)	1 (2)
L-Lyxose	26 (21)	25 (61)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
L-Galactonic acid δ-lactone	112 (92)	32 (78)	80 (99)	43 (98)	31 (100)	32 (100)	42 (98)

[a] Substrates are those showing differences in oxidation rates between *E. coli* subgroups. Differences of particular interest are shown in boldface.

positive tests in which an *E. coli* O157:H7 strain produced a substance which was active against OBGS lineage 1 strains as those in which such a strain produced a substance active against OBGS lineage 2 strains.

When the results are examined from the perspective of which strains were more frequently inhibited, the results for the two main subgroups are similar, with 50% of the sensitive test strains being commensal or K-12 *E. coli* strains and 50% being *E. coli* O157:H7. It was noted that 16 commensal strains and two K-12 strains were responsible for 51 and 33 sensitive tests, respectively, compared to 44 *E. coli* O157:H7 strains being responsible for the other 84 sensitive tests. Seven commensal *E. coli* strains and 18 *E. coli* O157:H7 strains were sensitive to more than one producer strain. These results can also be analyzed by subgroup. Of interest is that both commensal and O157:H7 *E. coli* strains are more likely to be inhibited by a substance produced by an *E. coli* O157:H7 strain than by a substance produced by a commensal strain.

Antibiotic sensitivity assays revealed that most *E. coli* strains were sensitive to the majority of antibiotics tested. However, 17 of the 81 *E. coli* O157:H7 strains and 11 of the 40 commensal strains were resistant to antibiotics (Table 2). Resistance to streptomycin, sulfamethoxazole, and tetracycline was most common. It is interesting that, when *E. coli* O157:H7 resistance patterns were examined by OBGS subgroup, a greater percentage of OBGS lineage 2 than of lineage 1 strains were resistant.

Individual fitness. The maximum individual growth rate was measured for 122 commensal and O157 *E. coli* strains in four different environments (Table 3). Mean growth rates for both groups were higher when cells were grown in the rich TSB medium than when they were grown in the minimal or rumen medium. Somewhat higher growth rates were also observed for cells grown under aerobic conditions. However, there were no significant differences in growth rates between the commensal and O157 strains under any of the four conditions. In addition, when the average growth rates for the different subgroups (cattle versus human strains and OBGS lineage 1 versus lineage 2) were compared for each growth condition, no significant differences were observed (data not shown). That is, for all conditions, the commensal strain group grew at the same rate as the O157 group did, and the cattle strain group and the human strain group grew at the same rate, as did both of the OBGS lineages.

TABLE 2. Antibiotic resistance of commensal *E. coli* and *E. coli* O157:H7

Antibiotic	No. of isolates (% of total) resistant to each antibiotic	
	Commensal strains (n = 41)	O157:H7 strains (n = 81)
Ampicillin	2 (5)	1 (1)
Kanamycin	2 (5)	3 (4)
Streptomycin	15 (37)	7 (9)
Sulfamethoxazole	14 (34)	10 (12)
Tetracycline	17 (42)	12 (15)

TABLE 3. Growth rates of commensal *E. coli* and *E. coli* O157:H7

Medium	Condition	Group	Avg μ ± SD
TSB	Aerobic	Commensal	1.21 ± 0.25
		O157	1.22 ± 0.10
	Anaerobic	Commensal	1.08 ± 0.23
		O157	1.05 ± 0.18
Minimal	Aerobic	Commensal	0.42 ± 0.12
		O157	0.42 ± 0.09
Rumen	Anaerobic	Commensal	0.35 ± 0.10
		O157	0.34 ± 0.09

DISCUSSION

Despite intense research efforts aimed at controlling and eliminating *E. coli* O157:H7, little is known about the means by which this organism spreads and persists in foods and the food production environment. We are interested in questions of how *E. coli* O157:H7grows and survives during its life cycle in the animal host and the external environment and what distinguishes these pathogens from their commensal counterparts that live as normal members of the primary and secondary habitats.

Data presented here support the hypothesis that *E. coli* O157:H7 strains, as a group, have a different carbon-source utilization pattern from that of commensal *E. coli* strains. There were 19 carbon sources that were differentially oxidized by *E. coli* O157:H7 than by commensal *E. coli* strains. When *E. coli* O157:H7 was first described, extensive biochemical analysis were performed on this new clone in search of phenotypic characteristics that would distinguish it from nonpathogenic *E. coli* strains (1, 44, 59). In addition to sorbitol fermentation, which was widely exploited for screening and identification of the new pathogen (34), raffinose, dulcitol, and rhamnose were also initially considered as potential discriminatory substrates (43). The use of the phenotypic microarray plates allowed us to expand the range of substrates examined and process samples in a high-throughput manner. Among the 19 carbon sources identified using the Biolog plates were sorbitol and dulcitol, as expected. It is important to note that the Biolog plates measure substrate oxidation and not sugar fermentation. This can be seen most dramatically in the sorbitol results for the 19% of the *E. coli* O157:H7 strains that were positive for sorbitol oxidation on the Biolog ES plate but negative for sorbitol fermentation when grown on sorbitol-MacConkey agar. *E. coli* O157:H7 strains were more likely to use dulcitol, sucrose, and L-galactonic acid δ-lactone than were their commensal *E. coli* counterparts. All other differentially used carbon sources were more frequently used by commensal *E. coli* than by *E. coli* O157:H7 strains. Thus, within the limits of the 95 carbon sources tested in this assay, distinct patterns of carbon source utilization exist for *E. coli* O157:H7 and commensal *E. coli* strains. These observations may be due to stochastic events or, alternatively, may be a result of exposure to different selective pressures.

In general, the commensal *E. coli* strains oxidized more substrates than the *E. coli* O157:H7 strains did. This was unexpected, considering the larger genome of the *E. coli* O157:H7 strains (7, 29, 39). Sequence analysis has revealed that *E. coli* O157:H7 codes for 1,632 proteins not found in *E. coli* K-12, only 131 of which are associated with virulence functions (29). We had expected that some of these non-virulence-associated *E. coli* O157:H7-specific proteins might serve to enhance the metabolic flexibility of the pathogenic strains. Our results showed, however, that *E. coli* O157:H7 strains were metabolically less diverse, based on carbon utilization patterns, than were the commensal strains tested.

Differences were also observed in the intraspecific competition patterns of *E. coli* O157:H7 and commensal *E. coli* strains. Intraspecific competition, especially the production of bacteriocins, has been well studied. Early work (47, 48) showed that a large percentage of *E. coli* strains both produce and are resistant to at least one bacteriocin and that both commensal and O157:H7 *E. coli* cells are capable of producing colicins (35, 36, 48). Despite extensive research on *E. coli* to characterize the production of and sensitivity to colicins under mitomycin C-induced laboratory conditions (19, 21, 35, 36, 47–49), little is known about the patterns of interaction under more naturalized settings or whether *E. coli* O157:H7 strains, as a group, demonstrate the same intra- and interspecific competition patterns as their commensal counterparts do. We therefore decided to characterize the nature of intraspecific *E. coli* interactions under noninduced conditions. Data from our experiments show that *E. coli* O157:H7 and commensal *E. coli* appear to have different competition patterns, with *E. coli* O157:H7 more likely to both produce and be sensitive to inhibitory agents when grown under standard laboratory conditions.

The inhibitory interactions observed in these experiments most likely represent bacteriocin-mediated interactions. Phage-mediated interactions were ruled out based on the zone morphology and the inability to propagate plaques. In addition, zones of clearing were still observed even after the supernatant was boiled before inoculation. Since many of the bacteriocin proteins are small (8), they are not easily denatured by heat. Finally, most of the bacteriocin plasmids in *E. coli* also contain genes coding for lysis of proteins, which are expressed during bacteriocin production (56). This would explain the zones of clearing around the disks despite the fact that live producer cells were also inoculated onto the plate. Thus, most of the zone-of-clearing interactions were suspected to have been due to colicin production by the producer strain, although the substances responsible for the clearing were not further characterized.

Overall, zones of clearing were observed in only 1% of the assays. As expected, this number is lower than the observations of Riley and Wertz that between 10 and 50% of all *E. coli* strains are producers of bacteriocins (49). This difference can be attributed to the fact that Riley and Wertz used mitomycin C to induce colicin production, whereas we performed our experiments under noninduced conditions. Within the positive tests, *E. coli* O157:H7 strains were much more likely to produce inhibitory substances than their commensal counterparts were. In 96% of the positive tests the producer strain was an *E. coli* O157:H7 strain, compared to only 4% of the tests showing a commensal strain as a producer. These results are in contrast to those of DeAlwis and Thomlinson (14), who examined colicin activity of commensal and pathogenic *E. coli* isolates from

pigs and found that the commensal strains produced a broad spectrum of colicins affecting the pathogenic strains but that the pathogenic strains produced only a few colicins, with a narrow spectrum. Our results showed that the *E. coli* O157:H7 supernatants were most commonly active against multiple strains, compared to only a single instance in which the commensal *E. coli* supernatant was active against more than one other strain. Furthermore, *E. coli* O157:H7 strains appear to have inhibited other *E. coli* O157:H7 strains as often as they inhibited K-12 and commensal *E. coli* strains. Thus, there does not appear to be any targeting of nonpathogenic strains by members of the *E. coli* O157:H7 group.

From the perspective of which strains were sensitive to inhibitory substances, both *E. coli* O157:H7 and commensal *E. coli* strains appeared to be moderately sensitive. In fact, the two K-12 strains were responsible for 33 of the 84 instances in which strains other than *E. coli* O157:H7 were inhibited. Since the K-12 strains were probably not very representative of wild commensal *E. coli* strains, the K-12 results were separated from those of other commensal *E. coli* strains. Thus, with the K-12 strains removed from the commensal category, 50% of the inhibited strains were *E. coli* O157:H7 strains, while only 30% of the inhibited strains were commensal *E. coli* strains. Therefore, the *E. coli* O157:H7 strains, as a group, were more likely than the commensal strains to be involved in production of and sensitivity to intraspecific inhibitory agents.

Minor differences were also observed in antimicrobial resistance patterns between the *E. coli* O157:H7 and commensal *E. coli* strains, with the former strains, in general, being more frequently resistant than the latter. Schroeder et al. (52) recently screened 361 *E. coli* O157:H7 strains for resistance to 13 antimicrobials also used in this study. Our overall susceptibility rates for *E. coli* O157:H7 strains of 61% are similar to the 63% overall susceptibility rates obtained by Schroeder's group. In most cases, strains in our study that demonstrated resistance were multiply resistant, such that one strain was resistant to more than one antibiotic. Our data also showed that commensal *E. coli* and *E. coli* O157:H7 populations carried resistance to a variety of antibiotics that involve different resistance mechanisms.

In both the primary and secondary habitats, different *E. coli* strains must compete with each other for (often limited) resources. The strains that are most fit would be expected to outcompete those that are less fit. We therefore reasoned that, unless emergence was due entirely to stochastic processes, there must be at least one niche in either the primary or secondary environments where the O157:H7 clone can outcompete other *E. coli* strains—that is, where the *E. coli* O157:H7 clone is more fit than its commensal *E. coli* competitors. To address this hypothesis, we designed a high-throughput 96-well assay to measure fitness of commensal and O157:H7 *E. coli* strains via growth rate, based on optical density readings over time. Fitness of O157:H7 *E. coli* and commensal *E. coli* strains was determined under four different conditions that mimic the environments that these organisms encounter in the primary, secondary, and laboratory environments. Our results showed that not only were there no significant differences in individual fitness between the O157:H7 and commensal *E. coli* strains tested but the growth rates of these groups were virtually identical in all four environments tested. These results were surprising, especially in light of the larger genome of the *E. coli* O157:H7 strains and their more frequent production of antagonistic agents—both of which could be expected to increase the metabolic load and thus decrease the fitness of the *E. coli* O157:H7 strains compared to that of the commensal *E. coli* strains (20, 26, 49, 56). Also, we had expected to find one environment where the *E. coli* O157:H7 strains were more fit than their commensal counterparts. However, the fact that *E. coli* O157:H7 and commensal *E. coli* strains have similar fitness values in the four environments tested here does not exclude the possibility that these strains would show a fitness differential in other environments. In fact, bacteria have been shown to have different fitness values in different environments, and environmental fitness has been shown to be influenced by a variety of factors, including metabolic load, stress, and nutrient availability (16, 15, 45, 57).

The underlying hypothesis for the research presented here is that there are indeed physiological and ecological differences between *E. coli* O1457:H7 and other *E. coli* strains and that we would observe differences in fitness between *E. coli* O157:H7 and commensal *E. coli* strains. The alternative hypothesis, that these two groups of *E. coli* were physiologically the same, shared identical ecological niches, and had identical fitness values in primary and secondary habitat environments, was considered unlikely for several reasons. First, according to the tenets of natural selection, genetic differences between two individuals that affect viability or reproductive success will increase or decrease the fitness of an individual relative to others in the population. Thus, different genotypes can be expected to have different fitness values (22, 23). Differences in growth rates have previously been demonstrated for *E. coli* in a variety of environments (15, 57).

The complete sequencing of two *E. coli* O157:H7 strains (29, 39) as well as a K-12 strain (7) and the enteropathogenic CFT073 strain (58) shows that *E. coli* O157:H7 strains are 270 kb larger than the enteropathogenic *E. coli* strain and 859 kb larger than the K-12 strain (29). While the K-12 strain most likely differs somewhat from its "wild" counterparts, it nonetheless provides us with a framework to examine some of the underlying genetic differences between *E. coli* O157:H7 and other *E. coli* strains. It is commonly accepted that genetic load may affect fitness. That is, if all other factors are equal, organisms with more DNA to copy will grow slower and thus be at a disadvantage against their competitors that have smaller genomes. This suggested that, in order for *E. coli* O157:H7 to be an efficient competitor (compared to other *E. coli* clones), these "extra" *E. coli* O157:H7-specific genes must also be important for the organism. That is, they must affect fitness values.

Finally, of the 1,632 *E. coli* O157:H7-specific proteins, only 131 were associated with virulence factors (29). If *E. coli* O157:H7 were transmitted directly from human to human, like its close relative *Shigella* sp., then the presence of the virulence genes alone may have been sufficient to account for the emergence of *E. coli* O157:H7—the virulence genes would have provided a fitness advantage to *E. coli* O157:H7, compared to its nonvirulent commensal *E. coli* counterparts, and allowed the pathogenic clone to outcompete other *E. coli* clones. Instead, epidemiological evidence has shown that *E. coli* O157:H7 outbreaks have been most commonly linked to a

primary food or water source, with secondary infections from person to person recorded only rarely. Additionally, if the virulence genes allowed *E. coli* O157:H7 to be reintroduced to cattle from a sick human, then the virulence genes alone may have been sufficient to account for the emergence of *E. coli* O157:H7. This pattern of transmission, however, has not been observed. Therefore, if the *E. coli* O157:H7-specific virulence genes alone are not sufficient to account for the emergence of the *E. coli* O157:H7 clone, then there may have been other differences between *E. coli* O157:H7 and other *E. coli* strains which gave the pathogen an advantage and allowed it to emerge. We suggest that it is likely that some of the remaining 1,501 *E. coli* O157:H7-specific proteins would influence how *E. coli* O157:H7 interacts in the primary and secondary habitats and would ultimately affect overall fitness values.

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