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# *Escherichia coli* O157:H7 and Other *E. coli* Strains Share Physiological Properties Associated with Intestinal Colonization▽†

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***Escherichia coli* isolates (72 commensal and 10 O157:H7 isolates) were compared with regard to physiological and growth parameters related to their ability to survive and persist in the gastrointestinal tract and found to be similar. We propose that nonhuman hosts in *E. coli* O157:H7 strains function similarly to other *E. coli* strains in regard to attributes relevant to gastrointestinal colonization.**

*Escherichia coli* is well known for its ecological versatility (15). A life cycle which includes both gastrointestinal and environmental stages has been stressed by both Savageau (15) and Adamowicz et al. (1). The gastrointestinal stage would be subjected to acid and detergent stress. The environmental stage is implicit in *E. coli* having transport systems for fungal siderophores (4) as well as pyrroloquinoline quinone-dependent periplasmic glucose utilization (1) because their presence indicates evolution in a location containing fungal siderophores and pyrroloquinoline quinone (1).

Since its recognition as a food-borne pathogen, there have been numerous outbreaks of food-borne infection due to *E. coli* O157:H7, in both ground beef and vegetable crops (6, 13). Cattle are widely considered to be the primary reservoir of *E. coli* O157:H7 (14), but *E. coli* O157:H7 does not appear to cause disease in cattle. To what extent is *E. coli* O157:H7 physiologically unique compared to the other naturally occurring *E. coli* strains? We feel that the uniqueness of *E. coli* O157:H7 should be evaluated against a backdrop of other wild-type *E. coli* strains, and in this regard, we chose the 72-strain ECOR reference collection originally described by Ochman and Selander (10). These strains were chosen from a collection of 2,600 *E. coli* isolates to provide diversity with regard to host species, geographical distribution, and electromorph profiles at 11 enzyme loci (10).

In our study we compared the 72 strains of the ECOR collection against 10 strains of *E. coli* O157:H7 and six strains of *E. coli* which had been in laboratory use for many years (Table 1). The in vitro comparisons were made with regard to factors potentially relevant to the bacteria's ability to colonize animal guts, i.e., acid tolerance, detergent tolerance, and the presence of the Entner-Doudoroff (ED) pathway (Table 2). Our longstanding interest in the ED pathway (11) derives in part from work by Paul Cohen's group (16, 17) showing that the ED pathway is important for *E. coli* colonization of the mouse large intestine. Growth was assessed by replica plating 88 strains of *E. coli* under 40 conditions (Table 2). These included two LB controls (aerobic and anaerobic), 14 for detergent stress (sodium dodecyl sulfate [SDS], hexadecyltrimethylammonium bromide [CTAB], and benzalkonium chloride, both aerobic and anaerobic), 16 for acid stress (pH 6.5, 6.0, 5.0, 4.6, 4.3, 4.2, 4.1, and 4.0), four for the ability to grow in a defined minimal medium (M63 glucose salts with and without thiamine), and four for the presence or absence of a functional ED pathway (M63 with gluconate or glucuronate). All tests were done with duplicate plates in two or three separate trials. The data are available in Tables S1 to S14 in the supplemental material, and they are summarized in Table 2.

**Detergent resistance.** SDS resistance is a convenient model for resistance to another powerful detergent, gastrointestinal bile salts (2, 7). Strain numbers (1 to 72) are from the ECOR reference collection (10). Aerobically, all strains grew well in SDS, both 1% and 5% SDS, except for ECOR strains 8, 9, and 63 and *E. coli* O157:H7 strains C503 and C535, which grew poorly. Anaerobically, 53 of the ECOR strains, one *E. coli* O157:H7 strain, and two of the lab-adapted *E. coli* strains grew

TABLE 1. *E. coli* strains used in this study

| *E. coli* strain (*n*) | Source |
|---|---|
| ECOR strains (72) | Thomas Whittman |
| Laboratory adapted (6) | |
| K-12 Davis | Paul Blum |
| CG5C 4401 | Paul Blum |
| K-12 Stanford | Paul Blum |
| W3110 | Paul Blum |
| B | Tyler Kokjohn |
| AB 1157 | Tyler Kokjohn |
| O157:H7 (10) | |
| FRIK 528 | Andrew Benson |
| ATCC 43895 | Andrew Benson |
| MC 1061 | Andrew Benson |
| C536 | Tim Cebula |
| C503 | Tim Cebula |
| C535 | Tim Cebula |
| ATCC 43889 | William Cray, Jr. |
| ATCC 43890 | William Cray, Jr. |
| ATCC 43888 | Willaim Cray, Jr. |
| ATCC 43894 | William Cray, Jr. |

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TABLE 2. Physiological comparison of 88 strains of *Escherichia coli*

| Growth medium or condition | Oxygen[c] | No. of strains with type of growth[b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ECOR strains ($n$ = 72) | | | | Laboratory strains ($n$ = 6) | | | | O157:H7 strains ($n$ = 10) | | | |
| | | Good | Poor | None | Variable | Good | Poor | None | Variable | Good | Poor | None | Variable |
| LB control[a] | Both | 72 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| 1% SDS | Aerobic | 69 | 3 | 0 | 0 | 6 | 0 | 0 | 0 | 8 | 0 | 0 | 2 |
| 5% SDS | Aerobic | 68 | 4 | 0 | 0 | 6 | 0 | 0 | 0 | 8 | 2 | 0 | 0 |
| 1% SDS | Anaerobic | 53 | 15 | 4 | 0 | 2 | 3 | 1 | 0 | 1 | 7 | 0 | 2 |
| 5% SDS | Anaerobic | 0 | 68 | 4 | 0 | 0 | 4 | 2 | 0 | 0 | 7 | 0 | 4 |
| CTAB[d] (all) | Both | 0 | 0 | 72 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 10 | 0 |
| 0.05% BAC | Aerobic | 3 | 11 | 58 | 2 | 0 | 2 | 2 | 2 | 0 | 0 | 9 | 1 |
| 0.2% BAC | Aerobic | 0 | 1 | 71 | 0 | 1 | 0 | 5 | 0 | 0 | 0 | 10 | 0 |
| 0.05% BAC | Anaerobic | 2 | 3 | 67 | 0 | 0 | 1 | 5 | 0 | 0 | 0 | 9 | 1 |
| 0.2% BAC | Anaerobic | 0 | 0 | 72 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 10 | 0 |
| pH 6.5 | Both | 72 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| pH 6 | Both | 72 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| pH 5 | Both | 70 | 2 | 0 | 0 | 6 | 0 | 0 | 0 | 9 | 0 | 0 | 1 |
| pH 4.6 | Both | 70 | 2 | 0 | 0 | 6 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| pH 4.3 | Aerobic | 14 | 0 | 1 | 57 | 3 | 1 | 2 | 0 | 3 | 2 | 0 | 5 |
| pH 4.3 | Anaerobic | 69 | 3 | 0 | 0 | 3 | 1 | 2 | 0 | 1 | 1 | 0 | 0 |
| pH 4.1 or 4.2 | Aerobic | 0 | 0 | 72 | 0 | | ND[g] | | | | | ND | |
| pH 4.0 | Both | 0 | 0 | 72 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 9 | 1 |
| M63 with supplement[e] | | | | | | | | | | | | | |
| Glucose | Aerobic[f] | 69 | 1 | 2 | 0 | 5 | 0 | 1 | 0 | 9 | 0 | 1 | 0 |
| Glucose | Anaerobic[f] | 70 | 0 | 2 | 0 | 5 | 0 | 1 | 0 | 9 | 0 | 1 | 0 |
| Gluconate | Both | 69 | 1 | 2 | 0 | 5 | 0 | 1 | 0 | 9 | 0 | 1 | 0 |
| Glucuronate | Aerobic | 68 | 2 | 2 | 0 | 5 | 0 | 1 | 0 | 9 | 0 | 1 | 0 |
| Glucuronate | Anaerobic | 69 | 1 | 2 | 0 | 5 | 0 | 1 | 0 | 9 | 0 | 1 | 0 |

[a] Eight LB controls were run, two for each set of LB experiments: SDS, CTAB, benzalkonium chloride (BAC), and pH stress.
[b] Growth was measured as either +++, +, or 0 (good, poor, and none, respectively), with +++ being the growth achieved on the LB control plates. "Variable" means that two or three replicates did not agree. All experiments were done at 37°C.
[c] "Anaerobic" refers to use of an Oxoid anaerobic chamber. Aerobic and anaerobic growth data are presented together when the results were identical and separately when the results were not the same or the anaerobic set had not been done. LB plates were measured after 1 (aerobic) or 2 (anaerobic) days, and the M63 plates were measured after 2 or 3 days.
[d] CTAB used at 0.05, 0.2%, and 0.4%.
[e] M63 defined medium (3) was supplemented with glucose, gluconate, or glucuronate, all at 0.2%.
[f] Identical results were obtained with and without 0.0001% thiamine.
[g] ND, not determined.

well on 1% SDS, but none grew well on 5% SDS (Table 2). It is reasonable that the bacteria grew better on SDS aerobically because SDS resistance is energy dependent; the cells lyse when they run out of energy (2). However, the results were very different with cationic detergents. None of the *E. coli* strains grew on LB agar containing CTAB (Table 2), regardless of the CTAB concentration employed (0.05, 0.2, or 0.4%). This sharp distinction between the effects of anionic and cationic detergents is in agreement with our earlier work on over 200 strains of *Enterobacter cloacae* (7) and our later work showing that *E. coli* MC4100 could tolerate a maximum of only 0.01% CTAB (12). Benzalkonium chloride, another cationic detergent, is less harsh; with 0.05% benzalkonium chloride 14 of the 72 ECOR strains grew aerobically and 5 of those 14 also grew anaerobically (Table 2). None of the *E. coli* O157:H7 strains grew on benzalkonium chloride (0.05 or 0.2%) either aerobically or anaerobically, but four of the six laboratory-adapted strains grew on 0.05% benzalkonium chloride aerobically.

**Acid resistance.** Acid stress experiments for the ECOR collection show that all of the strains grew well, both aerobically and anaerobically, at pH 4.6 to 6.5 except for strains 8 and 9, which grew poorly at pH 4.6 and 5.0 (Table 2). Similarly, none of the strains grew at pH 4.0, 4.1, or 4.2. Results at pH 4.3 were highly variable, even with three trials. We conclude that, within the limitations of our replica plating method, pH 4.3 is the acidic threshold of growth for the strains examined, except for strains 8 and 9, for which it is somewhat higher. Our results with the 10 *E. coli* O157:H7 strains were virtually identical (Table 2). Our data are in agreement with the work of Lin et al. (9), who found the minimum growth pH of *E. coli* to be pH 4.4, and of Large et al. (8), who suggested that *E. coli* O157:H7 is not more acid resistant than are other *E. coli* strains. These data are, of course, subject to the caveat that growth in acid is not the same as survival in acid (9).

**ED pathway.** Only four strains (ECOR 29 and 52, MC1061, and the known auxotroph AB1157) could not grow on M63 with glucose. We did not identify the auxotrophic requirement(s) for these strains. The remaining 84 strains grew well under both aerobic and anaerobic conditions, with the curious exception of ECOR strain 20, which grew much better anaerobically than aerobically with all three carbon sources. In no case did the addition of thiamine permit growth or enhance growth. Significantly, all strains which grew on M63 with glucose also grew on M63 with gluconate or glucuronate. Thus, all strains of *E. coli* have a functional ED pathway. The initial view that *E. coli* possessed the ED pathway was based on only three strains of *E. coli* (5), later increased to 24 strains (18). Clearly, sugar acid metabolism via the ED pathway is important for growth of *E. coli* in intestinal habitats (11).

**Conclusions.** The major conclusion from this study is that the 10 strains of *E. coli* O157:H7 examined are equivalent to the 72 strains of the ECOR collection with regard to a series of in vitro physiological parameters selected because of their relevance to colonization of animal gastrointestinal tracts. Perhaps significant differences would have been found if we had chosen further in vitro tests, and perhaps our in vitro tests are not directly applicable to in vivo colonization. Nevertheless, the results presented show significant similarities between *E. coli* O157:H7 and other *E. coli* strains. Often *E. coli* O157:H7 control measures are primarily targeted to reducing or eliminating an *E. coli* serotype that is thought to be significantly different from those of other commensal *E. coli* strains. Even though *E. coli* O157:H7 can colonize the human gastrointestinal tract and be highly infectious to humans, it does not usually affect the health of other mammals, including cattle, which commonly carry *E. coli* O157:H7 as a normal part of their fecal flora. Thus, we suggest that strategies to address *E. coli* O157:H7 in nonhuman mammals should not be viewed as eliminating a pathogen but rather should be geared toward containing or controlling a naturally occurring commensal organism.

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