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Identification of Coccoid *Escherichia coli* BJ4 Cells in the Large Intestine of Streptomycin-Treated Mice

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***Escherichia coli* BJ4, a rat isolate, was used to examine the growth and differentiation of the microorganism in its natural habitat, the intestine. Growth of *E. coli* BJ4 in the large intestine of streptomycin-treated mice was compared with its growth in laboratory media. By a number of methods, it was shown that *E. coli* BJ4 differentiates, during growth in the intestine, into two distinct populations, one that has the characteristics of the laboratory-grown strain and one that appears as a coccoid cell. Furthermore, it was shown that there is a natural selection for the coccoid-type cell in the intestine, while in laboratory media growth of rod-shaped *E. coli* BJ4 is enhanced.**

The large intestine of humans and animals is colonized by a vast variety of bacteria. Colonic contents yield approximately 10^{12} bacteria per g, with a diverse repertoire of at least 400 species. The bacterial populations involved consist of hundreds of species, which for any given mammalian host interact in such a way as to achieve a relatively stable numerical balance (3, 9). A consequence of the stability of the gut ecosystem is that introduced and/or foreign organisms have difficulty in establishing in the intestine. The term colonization resistance was introduced to describe this phenomenon (26). Antibiotic treatment of animals has been shown to increase their susceptibility to colonization by exogenous antibiotic-resistant, gram-negative bacteria in general (10, 11). A number of antibiotic-treated, gnotobiotic, and germfree animal models have been used to overcome colonization resistance for studies of colonization of the intestine by a particular microorganism (7, 8, 20, 31).

Our present knowledge of regulation and growth of the intestinal flora, both in its establishment and prevention of bacterial overgrowth, is mainly composed of scattered information that needs to be interrelated into a coherent understanding of ecology and function. This information has mainly been derived from in vitro models and assays, which unfortunately do not truly reflect mechanisms of in vivo interactions. Batch cultures of fecal suspensions and continuous flow cultures have been developed in attempts to mimic the intestinal ecosystem (4, 13, 14, 27).

The aim of the present investigation was to examine the growth and differentiation of an *Escherichia coli* strain in its natural habitat, the mouse intestine, and compare it with growth in laboratory media. *E. coli* BJ4, used in this study, has previously been studied in vivo in norfloxacin-treated pigs and in vitro in a continuous flow system, and it was found to be able to colonize and proliferate in both systems (21). For our studies, the streptomycin-treated mouse model was chosen. The streptomycin treatment was found to eliminate the gram-negative flora from the gut, leaving almost intact the gram-positive flora and the strict anaerobes (20, 29, 30). The value of this model lies in its ability to simulate competitive interactions between bacterial strains in the natural environment of *E. coli* strains.

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We were able to show that *E. coli* BJ4 differentiates in vivo into two populations, one that has the characteristics of a strain grown in laboratory media and one that consists of coccoid cells which grow slowly. Eventually, over time, the *E. coli* BJ4 population found in the streptomycin-treated mouse large intestine is dominated by the coccoid *E. coli* cells.

MATERIALS AND METHODS

Bacterial strain and growth media. *E. coli* BJ4 was isolated from a healthy Wistar rat at the Institute of Toxicology, National Food Agency, Copenhagen, Denmark, and kindly provided to us. The original isolate of the strain was serotyped as rough:K−:H2 (21), whereas the isolate that was given to us was of serotype rough:K−:H−. It seems that the strain has adapted to growth in laboratory media and thereby lost its ability to produce flagella. The strain was found to produce type 1 fimbriae, and it harbors two unidentified plasmids of high molecular weight.

Throughout this work a streptomycin-resistant isolate of *E. coli* BJ4 was used. *E. coli* BJ4 Str^r was obtained by cultivating the strain on Luria-Bertani (LB) agar plates (Difco) supplemented with 100 μg of streptomycin (Sigma) per ml. The streptomycin-resistant isolate was found to be identical to its parent in respect to biochemical reactions and serology.

Bacterial cells were always grown in the presence of 100 μg of streptomycin per ml. The following media were used: minimal medium, LB broth (Difco); nonselective plates, 5% blood agar (Statens Seruminstitut; SSI 261003); selective plates for gram-negative rods, blue plates (SSI 261009) and MacConkey agar (Difco).

Determination of growth rates was performed in LB medium and minimal medium supplemented with either Casamino Acids, glucose, glycerol, succinate, or acetate.

Serotyping and biotyping. Serotyping was performed as described by Ørskov and Ørskov (23). Flagellum production was tested at 18 and 37°C. The *E. coli* biotype was determined by fermentations in the following 22 carbohydrates: glucose, maltose, lactose, saccharose, cellobiose, trehalose, sorbose, melibiose, arabinose, raffinose, xylobiose, galactose, rhamnose, fructose, mannose, dulcitol, sorbitol, mannitol, salicin, inositol, glycerin, and adonitol.

Biochemical reactions of the strains were tested by Gram staining; indole production; and negative reactions in KCN, gelatin, malonate, Voges-Proskauer, and urease tests.

Colonization experiments. Six- to eight-week-old, outbred albino female mice (Ssc:CF1; Statens Seruminstitut) were used for colonization experiments. The colonizations were performed as previously described (20). Briefly, the mice were given sterile water containing 5 g of streptomycin sulfate per liter. After 24 h, food and water were removed overnight and a 1-ml suspension of bacteria (10^{10} CFU/ml) in 20% sucrose was given to the mice per os. After drinking the bacterial suspension, the mice were returned to a normal diet and streptomycin-containing water. Fecal samples were collected, homogenized, diluted, and plated on relevant media. During the colonization experiments there was one mouse per cage and cages were changed daily. A colonization experiment typically lasted 3 weeks. Each series of experiments included three individually caged mice, and each experiment was performed at least twice with essentially identical results.

Cecal mucus and cecal content isolation. Twenty-four hours prior to mucus isolation, six mice (see above specifications) were given drinking water containing 5 g of streptomycin per liter. The mice were sacrificed, and the ceca were removed and placed in sterile petri dishes. Cecal contents and crude cecal mucus were isolated as described by Cohen et al. (6). Briefly, ceca were nicked, and cecal contents were carefully emptied in a petri dish. Ceca were then washed with HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid)-Hanks buffer (pH 7.4), and mucus was scraped off with a rubber spatula.

Specific isolation of *E. coli* BJ4 cells from a fecal suspension. Fecal samples were collected throughout the colonization experiment from each mouse and treated individually. The samples were diluted 10^2 times and left to settle for 10 min at 4°C. D-Mannose was bound covalently to Sepharose beads as previously described (15). To 2 ml of the fecal suspension, 200 μl of mannose-Sepharose beads was added and incubated at 4°C for half an hour to allow type 1-fimbriated bacteria to bind to mannose molecules on the surface of the beads. Free bacteria, not bound to the beads, were washed out, 0.5% sodium dodecyl sulfate (SDS) was added, and incubation was continued for 15 min at 4°C under gentle agitation. Mannose-Sepharose beads were pelleted by low-speed centrifugation. The supernatant contained only type 1-fimbriated bacteria, which were bound to D-mannose. The purity of the supernatant for *E. coli* BJ4 was checked by plating on nonselective and selective media. It was confirmed by growth experiments that the strain was unaffected by the mild SDS treatment (data not shown).

Flow cytometry. Pure cultures and bacteria isolated from feces of animals colonized with *E. coli* BJ4 were run on a flow cytometer (Skantron) to determine cell size distribution and DNA content per bacterial cell (5). Bacterial cells were fixed in 70% ethanol–10 mM Tris (pH 7.4) and stored at 4°C until staining. All samples were stained just before being applied to the flow cytometer. Stored cells were centrifuged and resuspended in 10 mM Tris (pH 7.4) containing 10 mM $MgCl_2$. DNA staining was performed by mixing equal parts of the resuspended cells with mithramycin (200 μg/ml) and ethidium bromide (40 μg/ml) in the same buffer.

Electron microscopy. Bacterial preparations were taken directly from a primary plate, i.e., one on which a fecal suspension was plated, or from an overnight culture and negatively stained with 2% ammonium molybdate (pH 7.0), on Formvar-coated copper grids by method A (2).

16S rRNA probes. Three probes specific for *E. coli* 16S and 23S rRNA were designed. The sequences of the probes are as follows: Ec 634–654, 5'-CAAGCTTGCCAGTATCAGA TG-3'; Ec 995–1026, 5'-CTCTGAAAACTTCCGTGGATG-3'; Ec 1531–1551, 5'-CACCGTAGTGCCTCGTCATCA-3'. The Ec numbers denote the position in the *E. coli* 16S and 23S rRNA to which the 3' end of the oligonucleotide probe hybridizes. The specificity of the probes was tested against the rRNA database (22) at the University of Illinois by using the probe checker program developed by Niels Larsen, Department of Microbiology, University of Illinois (unpublished data). In order to visualize the hybridized cells the probes were labelled with x-rhodamine (Molecular Probes) (25). The probes were purified by fast protein liquid chromatography, using a PepRPC HR5/5 column (Pharmacia, Uppsala, Sweden).

Whole-cell rRNA hybridization directly on fecal suspensions. One milliliter of a 10^2 dilution of a fecal suspension was centrifuged, and bacterial cells were fixed in 3% paraformaldehyde overnight at 4°C (1). After being washed in phosphate-buffered saline with 0.1% Nonidet P-40, the cells were stored at 4°C in 50% ethanol–10 mM Tris (pH 7.2)–0.1% Nonidet P-40 until further examination. Prior to hybridization, cells were bound to six-well gelatin-coated slides. The attached cells were dried by sequential washes in 50, 80, and 100% ethanol (3 min each). Following the ethanol series, 9 μl of hybridization buffer (40% formamide, 0.9 M NaCl, 20 mM Tris [pH 7.2], 0.1% SDS) containing 20 ng of probe was added to each well. The cells were hybridized for 4 to 16 h in a moisture chamber, washed twice for 15 min at 37°C with the hybridization buffer without probe, once in 0.9 M NaCl–20 mM Tris (pH 7.2)–0.1% SDS for 15 min at room temperature, and finally rinsed in water (1).

Epifluorescence microscopy and image analysis. A Carl Zeiss Axioplan microscope equipped with epifluorescence optics, a 100-W mercury lamb, and filter set 14 for x-rhodamine was used to visualize the hybridized cells. The microscope is attached to a 12-bit cooled slow-scan CCD camera (Photometrics, Tuscon, Ariz.). Images were captured by the software pmis (Photometrics) running on a personal computer (IBM).

RESULTS

Colonization of the mouse large intestine by *E. coli* BJ4. *E. coli* BJ4 colonizes the large intestine of streptomycin-treated mice at levels of 10^8 to 10^9 CFU/g of feces. When fecal samples were plated on streptomycin-containing medium selective for gram-negative bacteria (blue plates supplemented with streptomycin), large lactose-fermenting colonies were observed, as expected for *E. coli*. In contrast, when fecal samples were plated on nonselective rich medium (blood agar plates supplemented with streptomycin) two types of colonies were seen, large rough and small compact (Fig. 1). Colonies of both types were picked for further characterization. Single colonies were reisolated and characterized by biochemical, serological, and molecular genetic means. Both isolates were gram-negative microorganisms; cells from the large colonies were rod shaped typical *E. coli* cells, whereas cells from the small colonies were smaller and almost spherical. Both isolates had the same serotype (rough:K−:H2:F1), the same biotype as determined by carbohydrate fermentations, the same biochemical reactions, and the same plasmid profiles (data not shown). Cell size, fimbriation, and flagellation were confirmed by electron microscopy (data not shown). It is worth noting that *E. coli*

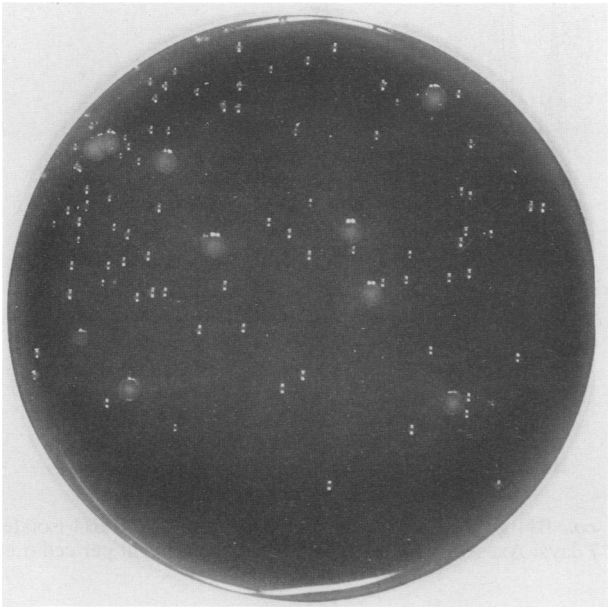

FIG. 1. *E. coli* BJ4 colonies growing on blood agar plates supplemented with streptomycin. The plate was streaked with diluted feces obtained from mice 7 days after challenge.

BJ4 regained its ability to produce flagella during its growth in the intestine. When originally isolated from a rat, the strain produced flagella of the H2 serotype. This ability was lost after growth in laboratory substrates.

The results presented above strongly suggest that *E. coli* BJ4 diverged into two phenotypes in the intestine, hereafter called BJ4-large and BJ4-small, according to their colony and cell size.

Growth in cecal mucus and cecal contents. Different cecal mucus and cecal content preparations (of approximately 1 ml) isolated from streptomycin-treated mice were inoculated with *E. coli* BJ4 (10^3 CFU), incubated at 37°C overnight, diluted, and plated on selective medium (MacConkey or blue plates) and nonselective medium (blood agar), both containing streptomycin. In all instances two types of colonies were seen on the nonselective medium, both at levels of 10^8 to 10^9 CFU/ml, while only large colonies (10^9 CFU/ml) were observed on MacConkey plates.

To eliminate the possibility that the differentiation of the *E. coli* cells is due to the presence of streptomycin in the gut, cecal mucus and cecal contents were isolated from non-streptomycin-treated mice. Cecal mucus and cecal contents were sterilized by UV light and inoculated with *E. coli* BJ4 (10^3 CFU/ml), and again the strain differentiated into two types of colonies.

Growth in laboratory media. The two variants of *E. coli* BJ4 isolated from mouse feces were cultivated in streptomycin-containing laboratory media for many generations. On plates, the small colonies remained small after repeated streaking, although single large colonies eventually showed up with frequencies expected for mutational revertants. In liquid media the population turned into cells forming large colonies after a few overnight incubations. Plating on MacConkey plates from either LB agar plates or LB liquid allowed normal growth of only large colonies. Bacterial cells isolated from the large colonies derived from fecal samples and from in vitro media behaved in all aspects like the original isolate of *E. coli* BJ4.

TABLE 1. Generation time of *E. coli* BJ4 when grown on two different laboratory media

Strain	Generation time (min) on	
	MM + 1% Casaa + 0.2% Glu[a]	MM + 0.2% Glu[b]
BJ4 original	26	44
BJ4-large	26	45
BJ4-small	42	82

[a] Minimal medium supplemented with Casamino Acids and glucose.
[b] Minimal medium supplemented with glucose.

E. coli BJ4-large and *E. coli* BJ4-small were grown in different media to measure their growth rates. The data in Table 1 show that *E. coli* BJ4-small has a generation time that is nearly twice that of *E. coli* BJ4-large in minimal medium supplemented with Casamino Acids and glucose and in minimal medium with glucose only. Similar results were obtained after growth in all the types of laboratory media described in Materials and Methods.

Cellular characteristics of *E. coli* BJ4 isolated directly from feces. It has previously been shown that type 1 fimbriae are expressed in the large intestine of the streptomycin-treated mouse (16). Type 1 fimbriae of *E. coli* bind specifically to mannose moieties. This characteristic of the fimbriated bacteria was used to isolate *E. coli* BJ4 cells selectively from feces (15) and to obtain pure suspensions without first having to culture the cells (see Materials and Methods).

In flow cytometry more than one cell parameter may be determined simultaneously for a large number of single cells, e.g., cell size and genomic content. In Fig. 2A, a histogram of a culture in stationary phase, after overnight growth in LB medium, is presented. The first peak represents the major cell population in stationary phase, which contains one chromosome, while the second peak represents the few cells that still have two chromosomes. In Fig. 2B, a typical population of *E. coli* BJ4 cells in fecal suspensions is shown. The distribution of the bacterial cells on the histogram indicates the presence of two cell populations with approximately the same number of cells but with clearly different cell sizes. It is easily seen that the small cells from the fecal samples are larger and contain more DNA than do stationary cells in vitro, and hence the small cells most likely are in the growth phase in vivo.

The two types of *E. coli* BJ4 cells were also characterized by using *E. coli*-specific oligonucleotide probes in connection with intracellular rRNA hybridization. Fecal samples were collected throughout the colonization experiment (approximately 4 weeks), diluted, fixed in paraformaldehyde, and stored until further characterization. Although the fecal samples consist of numerous bacterial species other than *E. coli*, the only cells that light up in the fluorescence microscope are those of *E. coli* BJ4, as the probe is highly specific to the *E. coli* rRNA. As a control, feces were sampled just before the mice were fed *E. coli* BJ4. This sample was free of *E. coli* cells. Twenty-four hours after the mice were fed BJ4 cells the fecal sample contained a uniform population of rod-shaped *E. coli* cells. After 3 days the fecal sample consisted of a mixed *E. coli* population with rod-shaped cells and a majority of coccoidal cells. When the bacterium had been in the intestine for over 2 weeks virtually all cells (within our detection limits) were coccoidal (Fig. 3). It can be seen in Fig. 3 that both cell types are dividing; thus, they are actively growing and not in stationary phase.

Colonization of the mouse large intestine with BJ4-large and

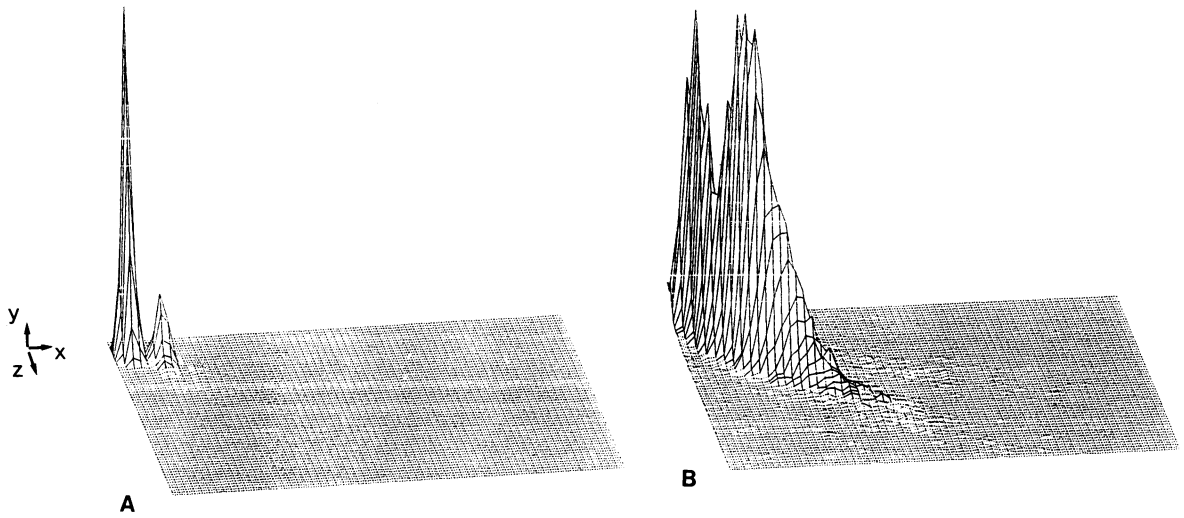

FIG. 2. Flow cytometer-generated histograms. (A) Stationary-phase *E. coli* BJ4 grown in laboratory medium. (B) *E. coli* BJ4 isolated specifically from a fecal suspension from mice colonized with BJ4 cells for 7 days. Axes: *y*, number of cells; *z*, scattered light per cell (i.e., cell size); *x*, DNA content (fluorescence signal).

BJ4-small. Mice were fed with overnight cultures of either BJ4-large or BJ4-small. Fecal samples were plated on blood agar plates in order to count large and small BJ4 bacterial cells. Twenty-four to forty-eight hours after the mice were fed *E. coli* BJ4-large the small colonies appeared in numbers similar to those of BJ4-large, i.e., 10^8 CFU/g of feces. Within a week the number of colonizing BJ4-small cells was 10^9 CFU/g of feces, while the number of BJ4-large cells was 2 orders of magnitude lower. At days 18 to 21 the numbers of BJ4-large cells had dropped to levels below our detection limits, while the number of BJ4-small cells remained at 10^8 CFU/g of feces throughout the colonization experiment. From fecal samples of mice colonized with BJ4-small, only small colonies were recovered, at levels of 10^8 to 10^9 CFU/g of feces, throughout the experiment.

DISCUSSION

In order to study the growth of *E. coli* strains in their natural habitat, here the large intestine, the streptomycin-treated mouse model was chosen. In this model the streptomycin treatment reduces the facultative anaerobic bacterial populations in the gut from about 10^8 CFU/g of feces to less than 10^2 CFU/g of feces, which is sufficient to overcome colonization resistance, thus allowing colonization by exogenous antibiotic-resistant strains. The obligate anaerobic populations, which are also shown to be strongly implicated in colonization resistance, do not significantly decrease and remain at approximately 10^8 CFU/g of feces (29, 30). The streptomycin treatment has no effect on the E_h, nutrient concentrations in mouse cecal contents, or intestinal motility. However, it causes a slight increase in water content and pH of cecal contents and a decrease in volatile fatty acids, thus facilitating colonization by exogenous strains (24).

When *E. coli* BJ4 colonizes the large intestine of the streptomycin-treated mouse it differentiates into two populations, BJ4-large and BJ4-small. By a number of classical, as well as molecular-based, tests we have shown that it is actually the same strain which during growth in the intestine has altered its growth characteristics. The colonies and the cells of BJ4-small are smaller than those of BJ4-large, which appears identical to the BJ4 strain which was introduced into

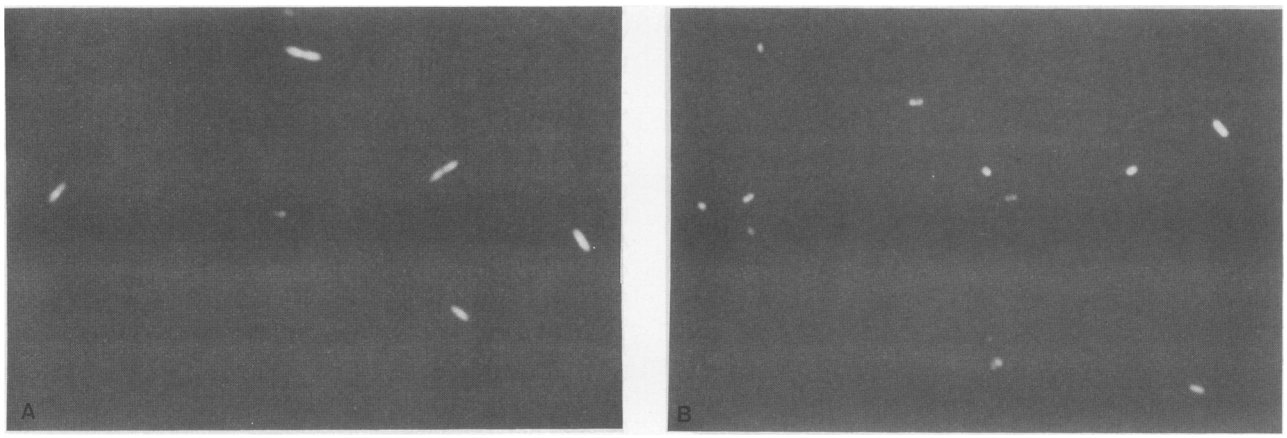

FIG. 3. rRNA hybridization with an *E. coli*-specific 16S rRNA probe on whole bacterial cells from a fecal suspension from mice colonized with BJ4 for 3 days (A) and for 7 days (B). BJ4-large and BJ4-small cells are clearly seen in the division state.

TABLE 2. Comparison of the two types of *E. coli* isolated from the large intestine of mice

Characteristic	BJ4 original	BJ4-large	BJ4-small
Fermentation of relevant C/H[a]	Yes	Yes	Yes
Indole	Positive	Positive	Positive
4-Nitro-phenyl-*meta*-D-glucopyranosiduronic acid	Positive	Positive	Positive
Gram stain	Negative	Negative	Negative
Serotype	R:K−:H−	R:K−:H2	R:K−H2
Fimbriae	Type 1	Type 1	Type 1
Plasmids	Two	Two	Two
Hybridization to *E. coli* 16S rRNA	Yes	Yes	Yes
Cell morphology	Rod	Rod	Coccoid
Colony morphology	Large, rough	Large, rough	Small, smooth
Growth rate	Fast	Fast	Slow
Sensitivity to MacConkey agar	No	No	Yes

[a] C/H, Carbohydrates listed in Materials and Methods.

the mice. BJ4-small cells multiply more slowly, are sensitive to growth on blue plates, and are relatively stable phenotypically. By repetitive growth on laboratory media BJ4-small reverts to the BJ4-large type. Differences as well as shared characteristics of the two types described are summarized in Table 2.

None of these changes were observed for any of the in vitro-grown BJ4-large cells. All media used were supplemented with streptomycin; therefore, the change from BJ4-large to BJ4-small does not seem to be caused by the streptomycin treatment of the animals. Growth in cecal mucus and cecal contents shows that the differentiation of *E. coli* BJ4 actually happens during its growth in vivo and not after the bacteria are shed from the intestine into feces. The role of mucus in supporting bacterial growth has been discussed previously by Wodolkowski et al. (28).

It has previously been reported that starved bacterial cells decrease in size and become smaller and spherical rather than rod shaped, whereas a nutritional upshift resulted in increased cell size (17). The size reduction is reported to be a continuous process during growth, culminating as the cells enter stationary phase (18). In the present study, BJ4-small colonies transferred to rich media still remained small. In addition, the flow cytometry data and rRNA hybridizations on cells in fecal samples showed that the *E. coli* BJ4-small cells are not starved or in stationary phase.

Our results indicate that the slow-growing variant BJ4-small is a more effective competitor in the intestinal environment. This is in agreement with a previous suggestion that growth rates of microorganisms present in the intestine must be much lower than those in a rich medium (12). Furthermore, results presented by Mikkola and Kurland (19) suggest that "domesticated" wild-type *E. coli* strains are fast-growing variants selected in the rich culture media commonly used in laboratories. All the above-mentioned results reflect the presence of selective pressures in the environment for a type of bacterial cells that are not observed in laboratory media.

Although the field of intestinal microecology is as old as medical microbiology, the growth and differentiation of bacteria in the intestine are still not understood. We have been able to show that growth in the mouse intestine induces stable changes in *E. coli* BJ4. It changes to a slow-growing coccoid cell. This is, of course, not necessarily true for all *E. coli* strains, but it should be recalled that there are major differences between the natural environment of these bacteria and the growth conditions in conventional laboratory media. Several other *E. coli* strains will be tested and compared during their growth in vivo and in vitro. Experiments are also currently being designed to identify the possible mutations that could be responsible for the above-described changes.

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REFERENCES

1. **Amman, R. I., L. Krumholz, and D. A. Stahl.** 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. **172**:762–770.
2. **Blom, J., G. A. Hansen, and F. M. Poulsen.** 1983. Morphology of cells and hemagglutinogens of *Bordetella* species: resolution of substructural units in fimbriae of *Bordetella pertussis*. Infect. Immun. **42**:308–317.
3. **Borriello, S. P.** 1986. Microbial flora of the gastrointestinal tract, p. 2–16. *In* M. J. Hill (ed.), Microbial metabolism in the digestive tract. CRC Press, Inc., Boca Raton, Fla.
4. **Borriello, S. P., and F. E. Barclay.** 1984. Colonization resistance to *Clostridium difficile* infection. Microecol. Ther. **14**:75–87.
5. **Boye, E., and A. Løbner-Olesen.** 1990. Flow cytometry: illuminating microbiology. New Biol. **2**:119–125.
6. **Cohen, P. S., J. C. Arruda, T. J. Williams, and D. C. Laux.** 1985. Adhesion of a human fecal *Escherichia coli* strain to mouse colonic mucus. Infect. Immun. **48**:139–145.
7. **Ducluzeau, R.** 1984. Microbial interactions in the digestive tract, p. 141–154. *In* M. E. Coates and B. E. Gustafsson (ed.), The germ free animal in biomedical research. Laboratory Animals, Ltd., London.
8. **Duval-Iflah, Y., P. Raibaud, and M. Rousseau.** 1981. Antagonisms among isogenic strains of *Escherichia coli* in digestive tracts of gnotobiotic mice. Infect. Immun. **34**:957–969.
9. **Finegold, S. M., V. L. Sutter, and G. E. Mathisen.** 1983. Normal indigenous intestinal flora, p. 3–31. *In* D. J. Hentges (ed.), Human intestinal flora in health and disease. Academic Press, New York.
10. **Freter, R.** 1955. The fatal enteric cholera infection in the guinea pig, achieved by inhibition of the normal enteric flora. J. Infect. Dis. **97**:57–65.
11. **Freter, R.** 1956. Experimental enteric *Shigella* and *Vibrio* infections in mice and guinea pigs. J. Exp. Med. **104**:411–418.

12. **Freter, R.** 1983. Mechanisms that control the microflora in the large intestine, p. 33–54. *In* D. J. Hentges (ed.), Human intestinal flora in health and disease. Academic Press, New York.
13. **Freter, R., E. Stauffer, D. Cleven, L. V. Holdeman, and W. E. C. Moore.** 1983. Continuous flow cultures as in vitro models of the ecology of large intestinal flora. Infect. Immun. **39:**666–675.
14. **Itoh, K., and R. Freter.** 1989. Control of *Escherichia coli* populations by a combination of indigenous clostridia and lactobacilli in gnotobiotic mice and continuous flow cultures. Infect. Immun. **57:**559–565.
15. **Klemm, P., K. A. Krogfelt, L. Hedegaard, and G. Christiansen.** 1990. The major subunit of *Escherichia coli* type 1 fimbriae is not required for D-mannose specific adhesion. Mol. Microbiol. **4:**553–559.
16. **Krogfelt, K. A., B. A. McCormick, R. L. Burgoff, D. C. Laux, and P. S. Cohen.** 1991. Expression of *Escherichia coli* F-18 type 1 fimbriae in the streptomycin-treated mouse large intestine. Infect. Immun. **59:**1567–1568.
17. **Kubitschek, H. E.** 1990. Cell volume increase in *Escherichia coli* after shifts to rich media. J. Bacteriol. **172:**94–101.
18. **Lange, R., and R. Hengge-Aronis.** 1991. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^S. J. Bacteriol. **173:**4474–4481.
19. **Mikkola, R., and C. G. Kurland.** 1991. Is there a unique ribosome phenotype for naturally occuring *Escherichia coli*? Biochimie **73:**1061–1062.
20. **Myhal, M. L., D. C. Laux, and P. S. Cohen.** 1982. Relative colonizing abilities of human fecal and K12 strains of *Escherichia coli* in the large intestines of streptomycin-treated mice. Eur. J. Clin. Microbiol. **1:**186–192.
21. **Nielsen, M. E., and J. Schlundt.** 1992. Use of norfloxacin to study colonization ability of *Escherichia coli* in in vivo and in vitro models of the porcine gut. Antimicrob. Agents Chemother. **36:**401–407.
22. **Olsen, G. J., N. Larsen, and C. R. Woese.** 1991. The ribosomal RNA database project. Nucleic Acids Res. **19:**2017–2021.
23. **Ørskov, F., and I. Ørskov.** 1984. Serotyping of *Escherichia coli*. Methods Microbiol. **14:**43–112.
24. **Que, J. U., S. W. Casey, and D. J. Hentges.** 1986. Factors responsible for increased susceptibility of mice to intestinal colonization after treatment with streptomycin. Infect. Immun. **53:**116–123.
25. **Stahl, D. A., and R. I. Amann.** 1991. Development and application of nucleic acid probes, p. 205–248. *In* E. Strackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, New York.
26. **Van der Waaij, D., J. M. Berghuis-de Vries, and J. E. C. Lekkerkerk-van der Wees.** 1971. Colonization resistance of the digestive tract in conventional and antibiotic treated mice. J. Hyg. **69:**405–413.
27. **Veilleux, B. G., and I. Rowland.** 1981. Simulation of the rat intestinal ecosystem using a two stage continuous flow culture system. J. Gen. Microbiol. **123:**103–115.
28. **Wadolkowski, E. A., D. C. Laux, and P. S. Cohen.** 1988. Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of growth in mucus. Infect. Immun. **56:**1030–1035.
29. **Wells, C. L., M. A. Maddaus, R. P. Jechorek, and R. L. Simmons.** 1988. Role of intestinal anaerobic bacteria in colonization resistance. Eur. J. Clin. Microbiol. Infect. Dis. **7:**107–113.
30. **Wilkins, T. D., and M. D. Appleman.** 1976. Review of methods for antibiotic susceptibility testing of anaerobic bacteria. Lab. Med. **7:**12–15.
31. **Yurdusev, N., M. Ladire, R. Ducluzeau, and P. Raibaud.** 1989. Antagonism exerted by an association of a *Bacteriodes thetaiotaomicron* strain and a *Fusarium necrogenes* strain against *Clostridium perfringens* in gnotobiotic mice and fecal suspensions incubated in vitro. Infect. Immun. **57:**724–731.