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Physiological State of *Escherichia coli* BJ4 Growing in the Large Intestines of Streptomycin-Treated Mice

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Growth rates of *Escherichia coli* BJ4 colonizing the large intestine of streptomycin-treated mice were estimated by quantitative hybridization with rRNA target probes and by epifluorescence microscopy. The ribosomal contents in bacteria isolated from the cecal mucus, cecal contents, and feces were measured and correlated with the ribosomal contents of bacteria growing in vitro at defined rates. The data suggest that *E. coli* BJ4 grows at an overall high rate in the intestine. However, when taking into account the total intestinal volume and numbers of bacteria present in cecal mucus, cecal contents, and feces, we suggest that *E. coli* BJ4 in the intestine consists of two populations, one in the mucus which has an apparent generation time of 40 to 80 min and one in the luminal contents which is static.

The animal intestinal tract harbors a vast number of bacteria representing a complex ecosystem in which the microorganisms are present without overgrowing the host but also without being flushed out by the host's intestinal activities, e.g., peristaltic movements and fluid flow. At least 400 to 500 different bacterial species are thought to be present at any time in the healthy human intestinal tract, and up to 10^{12} bacteria are found per g of feces (3, 9). One question of interest is whether the intestinal flora grows as a homogeneous population at a low rate or as a heterogeneous population containing fast, slow, and nongrowing organisms.

Accurate calculations of the rates of bacterial proliferation in the intestine have so far been represented only by average estimates at the level of populations. For example, the growth rate of *Escherichia coli* has been estimated in vivo, i.e., in the mouse intestine, by radioisotope techniques (8), by dilution by growth of a nonreplicating genetic marker (17, 25), and simply by counting the number of viable cells (12, 14). With these techniques, generation times from 30 min to 40 h have been estimated for *E. coli* (12, 14, 16, 36). Also, continuous-flow cultures have been developed to mimic bacterial interactions in the gut and estimate overall growth rates (13, 27). These systems, however, do not reflect the physiological conditions in the gut, where entrapping of the bacteria in the mucus gel plays an important role. Furthermore, bacterial cell morphology, protein profiles, and growth physiology have been found to be distinct during growth in the intestine when compared with growth in laboratory media (22, 33).

We have previously found that in test tubes *E. coli* BJ4 is rod shaped, appears as large cells, and has a great potential for fast growth; however, soon after colonization of mice, *E. coli* BJ4 differentiates into a coccoid morphology, the so-called small variant, which grow more slowly than the rod-shaped *E. coli* cells in aerated test tubes (22). Here, we continue our investigation of the growth physiology of *E. coli* BJ4 present in its natural environment, the large intestine. Bacterial growth rates can be estimated from the cellular RNA and/or DNA contents, since the cellular RNA content in particular is strongly dependent on the growth rate (4, 19, 30, 37).

Recently developed methods based on hybridization to whole cells with fluorophore-labelled oligonucleotide probe targeting the rRNA and epifluorescence microscopy coupled to digital image analysis allow estimations of the concentration of rRNA in single cells. The ribosomal contents of the bacteria isolated from the environment may then be correlated with the ribosomal contents of bacteria growing at defined rates (6, 34). In this study, we describe the growth physiology of *E. coli* BJ4 in the large intestine of streptomycin-treated mice.

MATERIALS AND METHODS

Bacterial strain and growth media. *E. coli* BJ4, Orough:K$^-$:A2:F1, was isolated from a healthy Wistar rat at the Institute of Toxicology, National Food Agency, Copenhagen, Denmark, and kindly made available to us. This strain and a streptomycin-resistant derivative were previously described in detail by Krogfelt et al. (22). Bacteria were always grown in the presence of 100 μg of streptomycin per ml. The following media were used: L broth, AB minimal medium (5), and 5% blood agar plates (Statens Seruminstitut, Copenhagen, Denmark; SSI 261003). As selective plates for gram-negative rods, blue plates (SSI 261009) and Bacto MacConkey Agar (Difco) plates were used.

Measurements of growth rates in laboratory media. Pure cultures were grown in the above-described media with different carbon sources added to AB minimal medium (5). Growth rates were measured by monitoring the optical density at 450 nm. Anaerobicity was obtained by blowing N_2 through the medium for 5 min prior to inoculation and for an additional 5 min after inoculation, after which the culture vessels were closed and left shaking very gently. Generation times are expressed in minutes or as specific growth rates, ln 2/hour, i.e., reciprocal hours.

Ribosomal efficiency measurements. The in vitro translation system for testing ribosomal efficiency was performed exactly as described by Ehrenberg et al. (7). Ribosomes and components were prepared and stored at −80°C (7, 18). The Michaelis constant (K_m), the catalytic rate constant (K_{cat} or V_{max}), and the second-order rate constant (R factor; number of amino acids captured per ribosome per second per mole of EF-Tu) were measured in standard assays and calculated by Eadie-Hofstee plot as described by Bohman et al. (2). All chemicals used were reagent grade from Sigma. Poly(U) was obtained from Pharmacia, Uppsala, Sweden; the radioactive amino acids were from Amersham, Amersham, United Kingdom.

Colonization experiments. Outbred albino female mice (Ssc:CF1, Statens Seruminstitut), 6 to 8 weeks old, were used for the colonization experiments. The colonizations were performed as previously described (29). Briefly, mice were given sterile water containing 5 g of streptomycin sulfate per liter. After 24 h, food and water were removed overnight; subsequently, 1 ml of a stationary-phase *E. coli* BJ4 culture (10^{10} CFU/ml) in 20% sucrose was given to the mice per os.

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After drinking the bacterial suspension, the mice were returned to their normal diet and sterile water containing streptomycin. Fecal samples were collected, homogenized, diluted, and plated. During the colonization experiments, the mice were individually caged and the cages were changed daily. Eight streptomycin-treated mice colonized with *E. coli* BJ4 were used for long-term colonization studies. Two mice each were sacrificed at days 4, 7, 11, and 20 after challenge. Studies on the initial colonization were done by inoculating the mice with 1 ml of *E. coli* BJ4 cells (10^6 CFU/ml). Two mice each were sacrificed at 1, 3, 5, 24, 96, and 120 h after challenge. From each mouse, samples of feces, cecal contents, and cecal mucus were taken for *E. coli* BJ4 CFU determination and hybridizations. Two conventionally raised mice and two streptomycin-treated mice not inoculated with *E. coli* BJ4 were used as controls. The experiment was repeated three times.

Bacterial cell smears from the gut. The colonized mice were sacrificed, and the ceca were removed and placed in sterile petri dishes. The ceca were nicked, and the cecal contents were carefully emptied into the petri dish. The ceca were then washed twice with *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-Hanks buffer (pH 7.4), and mucus was scraped off with a rubber spatula. Cecal contents, cecal mucus, and fecal samples were diluted 100-fold. Then, 1 ml was removed and fixed for rRNA hybridizations. Further dilutions were made and plated on blood agar plates for determination of viable counts of *E. coli* BJ4. All manipulations were completed as quickly as possible.

Specific isolation of *E. coli* BJ4 from the gut. *E. coli* BJ4 was specifically isolated from cecal mucus smears either with mannose-coated Sepharose beads (22) or by immunomagnetic separation with Dynabeads M-280 sheep anti-rabbit immunoglobulin G (Dynal 112.03). The Dynabeads were coated with specific antibodies against type 1 fimbriae raised in rabbits (21). Separation was carried out in a Dynal MPC-M magnetic particle concentrator, and bacterial cells were released from the beads by the addition of NaCl to 10%.

Fixation of bacterial cells. Cells isolated from cecal contents and cecal mucus, as well as cells from laboratory cultures, were fixed in 3% paraformaldehyde as previously described (35). Fixed cells were stored at −20°C in storage buffer (50% ethanol, 10 mM Tris [pH 7.5], 0.1% Nonidet P-40) until use.

Oligonucleotide probe. Probe EC 1531 (5′-CACCGTAGTGCCTCGTCAT CA-3′), specific to *E. coli* 23S rRNA, was labeled with lissamine rhodamine B sulfonyl (Molecular Probes, Eugene, Oreg.) and purified by reverse-phase liquid chromatography as previously described (35).

Whole-cell hybridization. Bacterial cell smears were hybridized on Teflon-coated slides as described previously (1, 35). The cells were immobilized to the slide by poly-L-lysine (Sigma Chemical, St. Louis, Mo.) coating (24). Hybridizations were carried out on the slides with 10 μl of solution I (35% formamide, 100 mM Tris [pH 7.5], 0.1% sodium dodecyl sulfate, 0.9 M NaCl) and 25 ng of probe and kept in a moisture chamber for 16 h at 37°C. Then the slides were rinsed in water and washed in 100 ml of prewarmed (37°C) solution I for 15 min and subsequently in 100 ml of prewarmed (37°C) washing solution II (100 mM Tris [pH 7.5], 0.9 M NaCl) for 15 min. The slides then quickly rinsed in distilled water and air dried.

After being stained with DAPI (4′,6′-diamidino-2-phenylindole; Sigma), the slides were rinsed in water, washed in solution I for 15 min at 37°C, and then transferred to 100 ml of solution II plus 0.625 μg of DAPI for 5 min at 21°C. The slides were subsequently transferred to 100 ml of solution II for 15 min at 37°C and finally rinsed in distilled water. For all quantitative purposes, the probe was labeled with lissamine rhodamine B and the hybridized cells were counterstained with DAPI.

Microscopy and image analysis. An Axioplan epifluorescence microscope (Carl Zeiss) was used to visualize the hybridizations. The microscope was equipped with a 100-W mercury lamp, and filter sets 1 (Carl Zeiss) and XF40 (Omega Optical, Brattleboro, Vt.) were used to visualize DAPI and lissamine rhodamine B, respectively. A 63×/1.25 Plan Neofluar Ph3 oil objective (Carl Zeiss) or a 63×/1.25 Plan Neofluar oil objective for differential interference contrast microscopy (Carl Zeiss) was used. A slow-scan charged coupled device (CCD) camera was used for capturing digitized images. The CCD camera was a CH250 camera (Photometrics, Tucson, Ariz.) equipped with a KAF 1400 chip (pixel size, 6.8 by 6.8 μm). The camera was operated at −40°C, and the chip was read out in 12 bits (4,096 intensity levels) at a rate of 200 kHz. The integration time for the CCD camera was 500 ms. Image analysis was done in 12 bits with PMIS software version 1.5 and version 2.11 (Photometrics), Cellstat (28), or NIH Image version 1.59 (available free at the ftp site: zippy.nimh.nih.gov). A DOS-based 486 computer was used as controller for the CCD camera, and a Macintosh Quadra 950 was used to run the Macintosh software. A Sun IPX computer was used to run Cellstat. Hybridized cells were automatically circumscribed by use of Cellstat, giving the cell size, fraction of dividing cells, and mean fluorescense intensity. In some cases, cells were also manually circumscribed by use of the NIH Image software.

In order not to bleach the lissamine rhodamine B prior to capturing images, cells were counterstained with DAPI, which was used for focusing the camera. At least three different images of 200 to 400 cells at each growth rate were quantified. The standard deviations of these measurements were 8 to 25%.

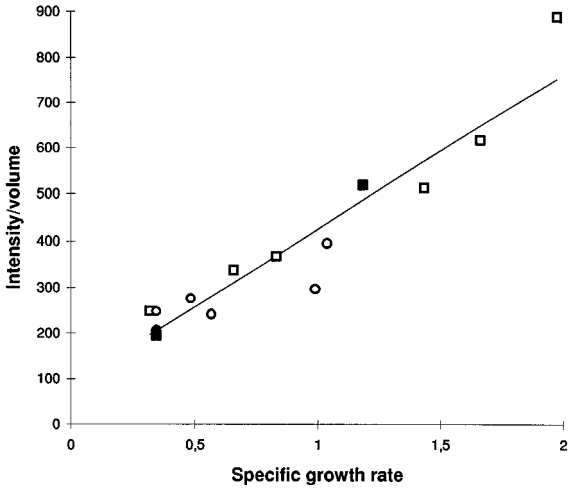

FIG. 1. Cellular content of ribosomes (pixel intensity per cell volume) inferred by whole-cell hybridization. Large (squares) and small (circles) *E. coli* BJ4 cells were grown aerobically (open symbols) in minimal medium supplemented with (from left to right) acetate, succinate, glycerol, glucose, and glucose with Casamino Acids and also in LB medium (fastest growth). Cells were also grown under anaerobic conditions (solid symbols) in two media: glycerol (slow growth) and glycerol with Casamino Acids (fast growth). See the text for details.

RESULTS

Growth rates and ribosomal efficiency in defined laboratory media. Pure-culture growth studies of *E. coli* BJ4 and the small-variant derivative (22) isolated from mouse feces after colonization of mice with the BJ4 strain were performed under aerobic conditions in AB minimal medium supplemented with either 0.2% glucose plus 1% Casamino Acids, 0.5% glucose, 0.5% succinate, 0.5% glycerol, or 1% acetate. These media supported specific growth rates of 1.66, 1.43, 0.83, 0.66, and 0.3 h^{-1}, respectively, for the large variant of *E. coli* BJ4 (the wild-type strain). The small variant of *E. coli* BJ4 was grown in the same media (except for acetate), supporting specific growth rates of 0.99, 0.57, 0.48, and 0.35 h^{-1}, respectively. In addition, rich medium (L broth) supplemented with 0.2% glucose, supporting specific growth rates of 1.95 and 1.04 h^{-1} for the large and small *E. coli* BJ4, respectively, was used (Fig. 1). Anaerobic growth took place in minimal medium supplemented with 0.5% glycerol (carbon source) and 0.5% sodium fumarate (electron acceptor), supporting specific growth rates of 0.34 h^{-1} for the large variant of *E. coli* BJ4 and 0.35 h^{-1} for the small variant of *E. coli* BJ4.

Ribosomes were purified from a laboratory ("domesticated") *E. coli* BJ4 culture and from two variant clones, i.e., the large and small *E. coli* BJ4, isolated from the feces of mice colonized with the domesticated type (22). All three types were grown in minimal medium plus 0.2% glucose and 1% Casamino Acids, supporting generation times of 28, 28, and 51 min, respectively. Ribosomal efficiencies were found to be almost identical in the three variants, the *R* factor being 1.05 ± 0.2 (standard deviation), 0.92 ± 0.2, and 0.87 ± 0.2, respectively (data not shown).

Ribosomal contents of *E. coli* BJ4 in defined laboratory media. The ribosomal contents of the coccoid (small) and rod-shaped (large) cells growing exponentially under aerobic conditions in various media supporting a range of growth rates were determined by whole-cell rRNA hybridization. Figure 1 shows how the cellular contents of ribosomes increase with the specific growth rate in the expected manner. Although the small BJ4 cells grew much more slowly than the large cells in

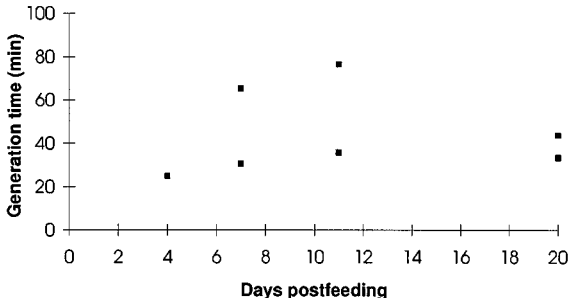

FIG. 2. Generation times in cecal mucus inferred by ribosomal hybridization. Each point represents the estimated doubling time of BJ4 in one mouse. On day 4, the two measurements were identical. The measured pixel intensities in bacteria isolated from the animals were correlated with the standard curve presented in Fig. 1, from which the doubling-time estimates were extracted.

the same medium, they showed an identical correlation with respect to growth rate and ribosomal contents. When the bacteria were cultured under anaerobic conditions in a minimal medium with glycerol as the carbon source and fumarate as the electron acceptor, the small coccoid and large rod-shaped *E. coli* BJ4 cells grew at approximately the same rate, i.e., 0.34 h^{-1}. Also, in these cells the ribosomal contents matched the standard-curve relationship between growth rate and ribosomal contents derived from growth under aerobic conditions (Fig. 1).

Thus, whole-cell hybridization and image analysis of the coccoid and rod-shaped derivatives of *E. coli* BJ4 grown aerobically and anaerobically showed identical relationships between growth rate and ribosomal contents, suggesting that the standard curve in Fig. 1 may be used for in situ determinations of the physiological activity of *E. coli* BJ4 regardless of cell shape and size (large or small) and the oxygen conditions in the intestine.

Ribosomal contents of *E. coli* BJ4 in the mouse intestine. Growth rates of *E. coli* BJ4 were estimated in samples from feces, cecal contents, and cecal mucus taken from mice colonized with the strain. Because of the limited focusing depth of a conventional microscope, we made no attempts to perform true in situ quantitative hybridizations; instead, we extracted the bacteria from the intestines and subsequently hybridized them as cell smears. Total bacterial populations were extracted from cecal contents, cecal mucus, and feces; fixed; spread in monolayers on hybridization slides; and hybridized as described in Materials and Methods. Digital images were captured for image analysis, and the mean signal intensity was measured (see Materials and Methods for details). By use of the standard curve in Fig. 1, the mean signal intensities could be converted into apparent growth rates.

Two mice each were sacrificed on days 4, 7, 11, and 20 after inoculation with *E. coli* BJ4, and samples were taken for hybridizations and plate counts. Numbers of CFU of *E. coli* BJ4 remained constant throughout the experiment at 2.6×10^9 and 1.1×10^8 CFU/ml in cecal contents and cecal mucus, respectively, and 1.9×10^9 CFU/g in the fecal samples. In Fig. 2, the average generation times of *E. coli* BJ4 in cecal mucus estimated from ribosomal contents determined by hybridization are presented. The apparent generation times of 30 to 80 min remained constant throughout the 20 days of investigation. In all animals, *E. coli* BJ4 cells had ribosomal contents within this range, indicating a high reproducibility of the growth conditions for the bacteria in the individual mice. We have performed many such determinations and have never found ribosome concentrations outside the range indicated here, so we are confident that the data in Fig. 2 are representative and statistically safe.

The initial stages of bacterial colonization were investigated by administering a smaller number of bacteria to the mice. Two mice each were sacrificed at 1, 3, 5, 24, 40, 96, and 120 h after challenge, and samples were taken for CFU determination and hybridizations. *E. coli* BJ4 colonization of the intestinal mucus increased rapidly, as shown in Fig. 3A. Quantitative measurements of rRNA hybridization showed that the ribosomal contents corresponded to generation times of 40 to 70 min in all samples taken between 1 and 120 h after challenge (Fig. 3B). Mucus and cecal contents from two streptomycin-treated unchallenged mice were hybridized with the *E. coli*-specific probe. No hybridization was observed, thus confirming the specificity of the probe used.

DISCUSSION

The major aim of the present studies has been to estimate the growth rates of *E. coli* when present in its natural environment, the intestinal tract of mammals. The streptomycin-treated mouse was used as the model animal, mainly because bacterial colonization of this animal model has been studied in great detail (11, 20, 29, 38) but also because previous investigations showed that *E. coli* may not be such a frequent colonizer of conventional animals (35) and it is very unlikely that an introduced *E. coli* culture would become established in a conventional animal. This, however, means that our conclusions may be true only for streptomycin-treated mice, and we are therefore planning future experiments with germ-free and conventional mice. For our studies, we chose to use *E. coli* BJ4 isolated from rat as the mouse colonizer, and in previous communications we have demonstrated that this strain colonizes streptomycin-treated mice very well (22), that the bacteria are found mainly in the mucus of the large intestine and the cecum

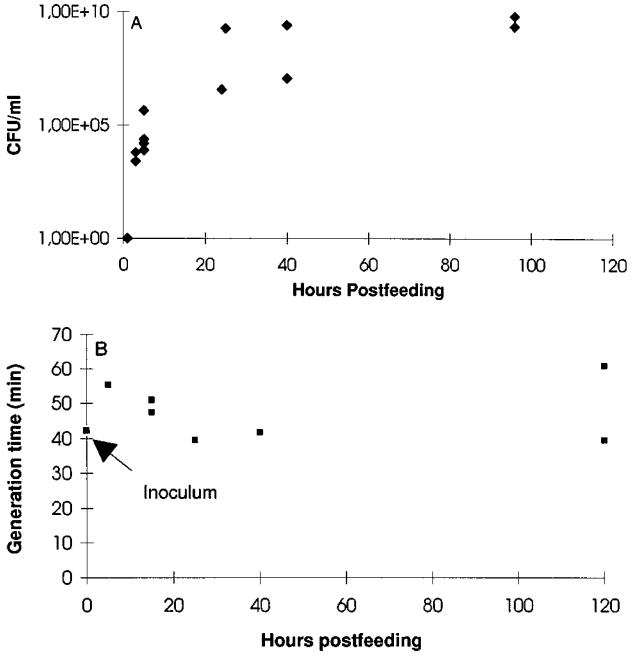

FIG. 3. Colonization of the mouse large intestine by *E. coli* BJ4 during the first 120 h of colonization of the mouse large intestine. (A) *E. coli* BJ4 CFU/ml of mucus. (B) Generation times in cecal mucus inferred by ribosomal hybridization. Each point represents measurements from one mouse.

(35), and that there is an apparent selection for variants of the strain which exhibit surprising phenotypes such as coccoid cell shape, slow growth in vitro, temperature sensitivity (42°C), and poor or no growth on MacConkey agar substrates (22; unpublished data).

The method of in situ rRNA hybridization in whole fixed cells has proven very useful in several complex environmental contexts for specific identification of single bacterial species. Moreover, the hybridization signal may be quantitated with the aid of digitalized video cameras (CCD cameras) coupled to the fluorescence microscope, and through such measurements ribosomal concentrations in bacteria can be determined (35). To convert hybridization data to specific growth rate estimates, a standard curve for the particular strain defining the correlation between growth rate and rRNA hybridization signals (fluorescence) is required. When deciding how to create a standard curve for the BJ4 strain, we had to take into consideration two important facts concerning the strain and the growth conditions in the mouse gut: (i) the strain apparently is present as two different types in the gut (the large, fast-growing type and the small, slow-growing type [as defined from test tube experiments]), and (ii) the intestinal mucus environment in which the bacteria live is most probably micro- or anaerobic. In addition, there are indications that *E. coli* strains isolated directly from humans (clinical isolates) grow more slowly than laboratory strains because of a selection in the gut for ribosomes with reduced efficiencies (26). The in vitro growth experiments performed with strain BJ4 were therefore designed so that all these features were taken into consideration.

In a series of growth experiments, the two variants of *E. coli* BJ4 were cultivated in media supporting generation times from around 30 to 150 min. The immediate conclusion from these measurements was that the small variant isolated from the mice grows more slowly than the normal laboratory strain in all tested media under aerobic conditions. The ribosome concentrations in the two cell types depend only on the actual growth rate, not on the specific medium used; in other words, all points related to the quantitative hybridizations fall on the same standard curve (Fig. 1). In the growth experiments carried out under anaerobic conditions, we chose to focus on a medium allowing anaerobic respiration (with fumarate as the electron acceptor), and again we compared the two variants of BJ4. Surprisingly, the lack of oxygen resulted in nearly identical growth rates for the two types, and in the many experiments we have performed under these conditions, there is a tendency toward slightly faster growth for the small, coccoid type. Thus, there is no reason to assume that the selected variant has a growth rate problem in the oxygen-poor mouse gut. When comparing the ribosome determinations obtained under these conditions with the rest of the data, it was seen that anaerobically growing BJ4 cells also harbor the number of ribosomes predicted from the standard curve. We therefore conclude from these growth experiments performed in vitro under carefully controlled conditions that for *E. coli* BJ4 and its slow-growing variants, there is only one standard curve which correlates cellular ribosome concentrations with growth rate and that it should therefore be used to convert ribosome concentrations to estimates of specific growth rates no matter where the cells are growing and what type of variant is present. A prediction from the conclusion drawn above is that the slow-growing variant harbors ribosomes with the same efficiency as does the fast-growing variant (if we assume that basically all ribosomes are engaged in protein synthesis under the conditions tested). To test this prediction, the ribosome efficiencies for laboratory-grown cells (BJ4 large), fast-growing BJ4 isolates from mouse feces, and slow-growing BJ4 cells from mouse feces were determined. The values found are nearly identical within the limits of the method, and we therefore suggest that the slow in vitro growth observed for mouse isolates of BJ4 is not caused by mutations in the ribosome as observed previously for human isolates of *E. coli* (26) but may be triggered by mutations or phase shifting in the gene(s) involved in central metabolic pathways. We should emphasize, however, that at present we have no positive indications concerning the actual loci involved.

Having defined the relationship between the growth rate of *E. coli* BJ4 and its ribosomal contents, it was possible to obtain estimates of cellular growth rates in situ from quantitative single-cell rRNA hybridization data. Bacterial cells isolated from the cecal mucus of long-term-colonized mice had rRNA contents corresponding to generation times of 30 to 80 min (Fig. 2). Before this steady state is reached, the bacteria must grow with a generation time of at least 90 min during the period of 5 to 24 h after challenge to achieve the observed increase in CFU (Fig. 3) if factors such as washout, mucosal turnover, and release of bacteria from the mucus layer are not taken into consideration. Hybridizations in *E. coli* BJ4 cells isolated from cecal contents and feces showed approximately the same rRNA content (data not shown), indicating similar doubling times. However, we observed that *E. coli* BJ4 did not grow in fecal pellets after excretion during a period of 24 h at room temperature (data not shown). Since the total mucosal population stays constant during long-term colonization, the mean retention time of bacteria in mucus must be equal to the mean growth rate of the mucosal population. If we assume that there is no (or only little) bacterial growth in the luminal contents and feces, that the apparent growth rate in mucus is 1 h (the average from Fig. 2), and that bacterial cells stay associated with the mucosal layer for 1 h, the minimum size of the bacterial population in mucus which can produce the 2.3×10^9 BJ4 cells counted in feces during 24 h is $2.3 \times 10^9/24 = 9.6 \times 10^7$. This correlates well with the estimated population size in mucus at a given time during colonization (the mucus volume is approximately 0.1 ml [5a]), which was found to be 1.1×10^7 CFU (Fig. 3A). Thus, the estimated generation time of 40 to 80 min for *E. coli* BJ4 growing in the mucus of the large intestine of the mouse is in agreement with the cell numbers if we assume that growth takes place mainly in mucus.

Our results are supported by previous findings showing that the human isolate *E. coli* F18 grows with a generation time of about 27 min in mouse intestinal mucus in vitro but does not grow in cecal contents or feces (11, 38). The main source of carbon and nitrogen was found to be a phospholipid, secreted by the host, which is abundant in intestinal mucus (20). Also, for a different bacterial species, *Yersinia enterocolitica*, it has been shown that the bacteria do not grow in cecal contents or feces of rabbits (31). Moreover, we have observed in the samples from the different compartments of the mouse gut that there are no dividing cells in cecal contents or feces, whereas dividing *E. coli* cells are found frequently in mucus (data not shown). Finally, it has been shown that the luminal contents of the pig intestine have an antibacterial effect (23). Therefore, we suggest that in streptomycin-treated mice, bacterial cells are proliferating mainly in the mucus layer whereas those in the cecal contents and in the feces do not grow at all or only for a few cell generations. Nevertheless, the bacteria isolated from the luminal contents and the feces had ribosome concentrations in the same range as those measured in the bacteria from mucus (data not shown).

On the basis of the above arguments, we propose the following interpretation of the data presented here. The high cellular concentrations of rRNA observed in bacteria isolated

from the intestinal lumen and feces indicate that the bacteria have been growing fast while associated with the mucosal layer and that the ribosomes needed to maintain this high growth rate stay intact even after cessation of bacterial growth. This would further indicate that once the bacteria are removed from the mucus, there is an abrupt cessation rather than a gradual reduction of the growth rate; if the latter were the case, a dilution of the ribosomal contents would have been expected, and this is obviously not the case.

Several other examples of bacteria having high concentrations of ribosomes despite the lack of growth have been reported recently (10, 15, 32). It thus seems common to bacteria, at least under some conditions, to maintain pools of stable ribosomes under conditions that are not favorable for active growth. Although there is no direct proof, we find that the data presented here, which show that the ribosomes in (nongrowing) bacteria isolated from feces are as active in vitro as are ribosomes from a growing laboratory culture, strongly suggest that the excess ribosomes are simply idle particles not engaged in protein synthesis with a potential to become engaged again if the external conditions allow it.

The concentration of rRNA in a bacterial cell, as measured by in situ hybridization, in general reflects the physiological state of the cell, either as it happens to be at the time of sampling the cell or as it was just prior to sampling. It is important to emphasize, however, that conversion of quantitative measurements of ribosome contents to estimates of cellular growth rates is reasonable only if other parameters such as cell size, division index, and DNA content are also monitored (28); in addition, it is always important to make thorough considerations about the specific environment in which the bacteria are analyzed, as we have attempted to do here in the context of growth possibilities for *E. coli* in the various compartments of the mouse gut. On the basis of such a mixture of parameter analysis and environmental considerations, we propose that the *E. coli* population in the mouse large intestine consists of at least two populations: one situated in the mucus layer and growing with an apparent generation time of 30 to 80 min, and one sloughed into the luminal contents and feces which contributes only a little to growth.

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