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Neither Motility nor Chemotaxis Plays a Role in the Ability of *Escherichia coli* F-18 To Colonize the Streptomycin-Treated Mouse Large Intestine

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***Escherichia coli* F-18, isolated from the feces of a healthy human in 1977, is an excellent colonizer of the streptomycin-treated mouse large intestine and displays normal motility and chemotaxis ability. A chemotaxis-defective derivative of *E. coli* F-18, *E. coli* F-18 CheA$^-$, and a nonflagellated derivative, *E. coli* F-18 Fla$^-$, were constructed. These strains were found to colonize the streptomycin-treated mouse large intestine as well as *E. coli* F-18 when mice were fed both *E. coli* F-18 and either the CheA$^-$ or Fla$^-$ derivative at high levels (10^{10} CFU of each strain per mouse) or low levels (10^4 CFU of each strain per mouse). Furthermore, *E. coli* F-18 lost motility and chemotaxis ability when grown in colonic or cecal mucus in vitro despite retaining the ability to synthesize flagella. Thus, it appears that neither motility nor chemotaxis plays a role in the ability of *E. coli* F-18 to colonize because this strain becomes functionally nonmotile upon growth in the streptomycin-treated mouse large intestine.**

Evidence is accumulating that the importance of motility, chemotaxis, or both in the ability of a flagellated intestinal bacterium to colonize the mammalian intestine depends on the microorganism, whether the host has a conventional microflora, is antibiotic treated, or is gnotobiotic, and whether the microorganism colonizes the small or large intestine. For example, motility/chemotaxis appears to be important in the ability of the obligate anaerobe *Roseburia cecicola* to colonize the conventional but not the gnotobiotic mouse cecum (17) and for *Vibrio cholerae* to infect the gnotobiotic mouse cecum (8) and ligated loops of conventional rabbit and gnotobiotic mouse small intestine (8). Moreover, motility also appears to be a colonization factor for *Campylobacter jejuni* in the lower small intestine, cecum, and colon of suckling mice (14) and in the conventional adult rabbit small intestine (3). However, motility/chemotaxis does not appear to be important in the ability of *Salmonella typhimurium* to colonize the large intestines of conventional (4) or streptomycin-treated (12) mice, nor does motility appear to be important in the virulence of the organism (4, 10). Here, we show that motility/chemotaxis also does not appear to be important in the ability of a human fecal *Escherichia coli* strain, F-18, to colonize the mouse large intestine.

MATERIALS AND METHODS

Bacteria. *E. coli* F-18 was isolated from the feces of a healthy human in 1977. A streptomycin-resistant (Str^r) and rifampin-resistant (Rif^r) derivative of *E. coli* F-18 is an excellent colonizer of the streptomycin-treated mouse large intestine (6). The strain produces type 1 pili and colicin V, and its serotype is rough:K1:H5 (11). *E. coli* F-18 (Str^r Rif^r Nal^r) is a spontaneous nalidixic acid-resistant (Nal^r) mutant of *E. coli* F-18 (Str^r Rif^r) which in cofeeding experiments colonizes the streptomycin-treated mouse large intestine as well as its parent. *E. coli* F-18 (Str^r Rif^r) will hereafter be referred to as *E. coli* F-18.

Nonchemotactic derivatives of *E. coli* F-18 (i.e., nonspreading on semisolid L motility agar and nontumbling but motile microscopically) were selected on L-agar plates containing tetracycline (10 μg/ml) after bacteriophage P1 cotransduction of a defective *cheA* gene and a closely linked Tn*10* insertion from *E. coli* RP1781 into *E. coli* F-18, as described previously (11). Tn*10* was lost from the nonchemotactic transductants at high frequency in the absence of tetracycline selection (90% loss in 10 generations); however, the tetracycline-sensitive segregants remained nonchemotactic. A spontaneous Nal^r mutant was selected from one such nonchemotactic *E. coli* F-18 segregant as described previously (6) and designated *E. coli* F-18 CheA$^-$. In cofeeding experiments, *E. coli* F-18 CheA$^-$ (Str^r Rif^r Nal^r) colonized the streptomycin-treated mouse large intestine as well as its nalidixic acid-sensitive CheA$^-$ parent. Furthermore, it grew in Davis broth minimal (Difco Laboratories, Detroit, Mich.) with glucose as the sole source of carbon, as does *E. coli* F-18. *E. coli* RP1781 does not grow in this medium.

Nonflagellated derivatives of *E. coli* F-18 were selected on L-agar plates containing streptomycin (100 μg/ml), rifampin (50 μg/ml), and kanamycin (40 μg/ml) after P1 transduction from an *E. coli* MC1000 strain that contains a kanamycin cassette in the *flhD* gene, making it nonflagellated. All kanamycin-resistant (Kan^r) colonies were Str^r Rif^r and nonmotile (i.e., nonspreading on semisolid L motility agar and showing only Brownian motion microscopically). One colony was selected for further work and was designated *E. coli* F-18 Fla$^-$. *E. coli* F-18 Fla$^-$ also grew in Davis broth minimal with glucose as the sole source of carbon. *E. coli* MC1000 does not grow in this medium.

Media and antibiotics. L broth was made as described by Revel (16). L agar is L broth containing 12 g of Bacto-Agar (Difco) per liter. MacConkey agar (Difco) was prepared according to package instructions. L motility agar is L broth containing 3.5 mg of Bacto-Agar per ml of water. Cecal mucus motility agar is cecal mucus (2 mg of protein per ml in *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES]-Hanks buffer, pH 7.4) containing 3.5 mg of

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Difco agar purified per ml of water. In some experiments, L motility and cecal mucus motility agars were supplemented with adenosine 3',5'-cyclic AMP (cAMP; Sigma Chemical Co., St. Louis, Mo.) to a final concentration of 2 mM.

Mouse colonization experiments. The method used to distinguish the relative colonizing abilities of *E. coli* strains in mice has been described in detail previously (6, 11). Briefly, after 1 day of being fed streptomycin sulfate in their drinking water (5 g/liter), three male CD-1 mice (5 to 8 weeks old) were starved from 18 to 24 h for food (Charles River Valley Rat, Mouse, and Hamster Formula) and water and fed either 10^{10} or 10^4 CFU each of the L-broth-grown *E. coli* strains to be tested in 1 ml of sterile 20% (wt/vol) sucrose, as described previously (6, 11). The mice drank the bacterial suspension almost immediately and were then given food and streptomycin-containing drinking water. The next day and at 48-h intervals, fecal samples, no older than 24 h, were collected, homogenized, diluted, and plated on selective media as described below. In all colonization experiments, plates were incubated at 37°C for 18 to 24 h. Colonizing ability was assessed by the level at which a strain persisted in feces. Each experiment was performed at least twice, with essentially identical results.

To differentiate between *E. coli* F-18 (Str^r Rif^r) and *E. coli* F-18 CheA$^-$ (Str^r Rif^r Nal^r), fecal samples were plated on MacConkey agar containing 100 μg of streptomycin sulfate and 50 μg of rifampin per ml and on MacConkey agar containing 100 μg of streptomycin sulfate, 50 μg of rifampin, and 50 μg of nalidixic acid per ml. The numbers of *E. coli* F-18 CheA$^-$ were determined directly from the plates containing nalidixic acid. The numbers of *E. coli* F-18 were determined each day by transferring by toothpick 50 colonies from the plates without nalidixic acid to plates containing nalidixic acid and determining the fraction of the total that was nalidixic acid sensitive. The numbers of *E. coli* F-18 CheA$^-$ per gram of feces calculated from the results of toothpick transfer agreed very well with the numbers determined from direct plating.

To differentiate *E. coli* F-18 (Str^r Rif^r Nal^r) from *E. coli* F-18 Fla$^-$ (Str^r Rif^r Kan^r), fecal samples were plated on MacConkey agar containing 100 μg of streptomycin sulfate, 50 μg of rifampin, and 50 μg of nalidixic acid per ml and on MacConkey agar containing 100 μg of streptomycin sulfate, 50 μg of rifampin, and 40 μg of kanamycin monosulfate per ml.

Microscopic observation of *E. coli* F-18 grown in intestinal mucus in vitro. Jejunal, cecal, proximal colonic, midcolonic, and distal colonic mucus preparations (2 mg of protein per ml) were prepared from male CD-1 mice (5 to 8 weeks old) in HEPES-Hanks buffer, pH 7.4, as described previously (6, 12, 19). *E. coli* F-18 in HEPES-Hanks buffer, pH 7.4, was inoculated into each mucus preparation at about 10^4 CFU per ml and incubated standing for 18 h at 37°C. *E. coli* F-18 routinely grew to about 10^8 CFU per ml in each preparation. Uninoculated mucus preparations remained bacteria free when examined microscopically. As a control, L broth was inoculated with 10^4 CFU of *E. coli* F-18 per ml. Routinely, *E. coli* F-18 grew to about 2×10^8 CFU/ml upon standing for 18 h at 37°C. Wet mounts were examined for motility by phase-contrast microscopy under oil at ×1,000 magnification.

Viable counts of *E. coli* F-18 in the intestinal mucus of streptomycin-treated mice colonized with *E. coli* F-18. Three streptomycin-treated mice were each fed 10^{10} CFU of *E. coli* F-18. Eight days later the mice were sacrificed, and the jejunal, cecal, proximal colonic, midcolonic, and distal colonic mucus of each mouse was scraped separately into 5 ml of HEPES-Hanks buffer, pH 7.4. Each preparation was then homogenized and plated on L agar containing 100 μg of streptomycin sulfate and 50 μg of rifampin per ml. The plate counts were then corrected to the 5-ml volume to determine the total *E. coli* F-18 CFU present in each undiluted mucus sample.

Spread of *E. coli* F-18 on L motility and mucus motility agars. Three *E. coli* F-18 colonies, isolated from the jejunum, cecum, proximal colon, midcolon, and distal colon of each streptomycin-treated mouse colonized for 8 days with *E. coli* F-18, were streaked on L agar containing streptomycin sulfate (100 μg/ml) and rifampin (50 μg/ml), incubated at 37°C for 18 h, transferred by toothpick to L motility agar, and incubated at 37°C for 18 h. At that time, the diameter of spread of each colony was measured in millimeters.

In another experiment, *E. coli* F-18 was freshly streaked as described above and transferred by toothpick to L motility, jejunal mucus motility, cecal mucus motility, proximal colon mucus motility, midcolon mucus motility, and distal mucus motility agars and to each of these motility agars containing 2 mM cAMP. Incubation was at 37°C for 18 h, after which the diameters of spread were measured in millimeters.

Motility of cecal mucus-grown *E. coli* F-18 suspended in L broth. *E. coli* F-18 grown in cecal mucus as described above was centrifuged at room temperature at 3,000 × *g* for 5 min, washed once in HEPES-Hanks buffer, pH 7.4, and suspended at the same concentration in L broth. Similarly, *E. coli* F-18 grown in L broth was centrifuged, washed, and suspended in fresh cecal mucus (2 mg per ml of protein in HEPES-Hanks buffer, pH 7.4) and in spent cecal mucus, i.e., cecal mucus centrifuged free of *E. coli* F-18 that had grown in it. Motility of *E. coli* F-18 in each preparation was observed microscopically as described above.

Electron microscopy. Bacterial suspensions in either L broth or cecal mucus were stained for 10 s with 1% phosphotungstic acid as described by Arthur et al. (1) on copper grids coated with Formvar and carbon. The samples were examined in a JEOL 1200 SX microscope.

Chemicals. All chemicals were reagent grade.

RESULTS

Relative colonizing abilities of *E. coli* F-18, *E. coli* F-18 Fla$^-$, and *E. coli* F-18 CheA$^-$. When *E. coli* F-18 (Str^r Rif^r Nal^r) and *E. coli* F-18 Fla$^-$ (Str^r Rif^r Kan^r) were fed simultaneously (10^{10} CFU each) to streptomycin-treated mice, both colonized the large intestine in equal numbers, i.e., approximately 5×10^7 CFU/g of feces (Fig. 1). Each day, several presumptive Fla$^-$ colonies were tested for motility on L motility agar, and all remained nonmotile. Both *E. coli* F-18 and *E. coli* F-18 CheA$^-$ also cocolonized the streptomycin-treated mouse large intestine, i.e., each at about 5×10^7 CFU/g of feces (data not shown). Several *E. coli* F-18 CheA$^-$ colonies were tested each day as described above, and all remained nonchemotactic.

To determine whether motility/chemotaxis might be important when small numbers of *E. coli* F-18 are fed to mice, streptomycin-treated mice were fed 10^4 CFU of *E. coli* F-18 and *E. coli* F-18 Fla$^-$. Both strains grew rapidly in the streptomycin-treated mouse large intestine and cocolonized at about 10^8 CFU/g of feces (Table 1). Again, essentially identical results were obtained when 10^4 CFU each of *E. coli* F-18 and *E. coli* F-18 CheA$^-$ was fed to streptomycin-treated mice (data not shown). *E. coli* F-18 Fla$^-$ and *E. coli*

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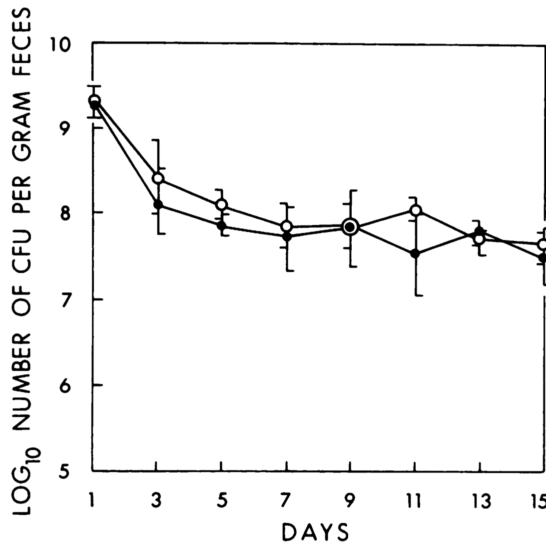

FIG. 1. Relative colonizing abilities of *E. coli* F-18 (Str^r Rif^r Nal^r) and *E. coli* F-18 Fla$^-$ (Str^r Rif^r Kan^r). Three streptomycin-treated mice were fed 10^{10} CFU each of *E. coli* F-18 (●), and *E. coli* F-18 Fla$^-$ (○). Symbols and bars represent the $\log_{10}$ mean number of CFU per gram of feces and standard error of the $\log_{10}$ mean, respectively.

F-18 CheA$^-$ colonies isolated from feces at all times during the duration of the experiments remained nonmotile and nonchemotactic, respectively.

Motility after growth in intestinal mucus. *E. coli* F-18 grows well in cecal mucus but poorly in cecal luminal contents in vitro (19), suggesting that *E. coli* F-18 derives its nutrition for growth in and colonization of the streptomycin-treated mouse large intestine from intestinal mucus. When *E. coli* F-18 was grown overnight in L broth, it was actively motile; however, when grown in mucus containing 2 mg of protein per ml isolated from either the cecum, proximal colon, midcolon, or distal colon, *E. coli* F-18 did not tumble and did not show any translational swimming movement. When grown in jejunal mucus, *E. coli* F-18 showed reduced but observable tumbling and swimming activity.

When *E. coli* F-18 was transferred by toothpick from L agar to L motility agar, it spread rapidly, indicative of normal motility/chemotaxis activity (9) (Table 2). In contrast, when *E. coli* F-18 was transferred by toothpick from L agar to mucus motility agar made from either jejunal, cecal, proximal colonic, midcolonic, or distal colonic mucus, it grew but did not spread (Table 2). Furthermore, addition of 2 mM cAMP to each of the motility agar plates did not result in increased spreading (Table 2). Typical spread of *E. coli* F-18 on L motility agar and cecal mucus motility agar is illustrated in Fig. 2.

TABLE 1. Relative colonizing abilities of *E. coli* F-18 and *E. coli* F-18 Fla$^-$ in streptomycin-treated mice fed 10^4 CFU of each strain

Day	Log_{10} no. of CFU/g of feces (mean ± SE)[a]	
	E. coli F-18	*E. coli* F-18 Fla$^-$
1	7.93 ± 0.92	8.01 ± 0.74
3	8.43 ± 0.36	8.57 ± 0.37
5	7.90 ± 0.28	8.04 ± 0.20

[a] Three streptomycin-treated mice were each fed 10^4 CFU of *E. coli* F-18 and 10^4 CFU of *E. coli* F-18 Fla$^-$. Fecal samples were diluted and plated as described in the text.

TABLE 2. Diameter of spread of *E. coli* F-18 on intestinal mucus motility agars

Type of motility agar	Diam of *E. coli* F-18 spread (mean ± SE)[a]	
	Unsupplemented	+2 mM cAMP
L	29.5 ± 0.5	29.5 ± 0.5
Jejunal	9.3 ± 0.8	11.5 ± 0.5
Cecal	8.0 ± 1.0	6.5 ± 0.5
Proximal colonic	8.3 ± 0.3	8.8 ± 0.8
Midcolonic	7.0 ± 1.0	7.8 ± 0.3
Distal colonic	9.5 ± 0.5	7.5 ± 0.5

[a] Spread of *E. coli* F-18 on the various motility agars was performed in duplicate, and measurements were made after incubation at 37°C for 18 h.

It might be argued that *E. coli* F-18 was nonmotile after growth in intestinal mucus because mucus is too viscous to allow normal tumbling and swimming. However, when *E. coli* F-18 grown in L broth was centrifuged and suspended in cecal mucus (2 mg/ml with respect to protein), it remained as motile as in L broth, suggesting that mucus viscosity was not responsible for the loss of *E. coli* F-18 tumbling and swimming activity when the cells were grown in cecal mucus. Furthermore, *E. coli* F-18 grown in L broth and suspended in spent cecal mucus also retained full tumbling and swimming activity.

Numbers and motility of *E. coli* F-18 in intestinal mucus throughout the intestine. Mucus isolated from the jejunum, cecum, proximal colon, midcolon, and distal colon of streptomycin-treated mice colonized with *E. coli* F-18 for 8 days was assayed for the numbers of *E. coli* F-18 in each preparation (Table 3). The cecal and proximal colonic mucus preparations contained about equal numbers of *E. coli* F-18 (Table 3). Twenty times fewer *E. coli* F-18 were found in the jejunal mucus preparation, and two to three times fewer were found in the midcolonic and distal colonic mucus preparations (Table 3). *E. coli* F-18 colonies isolated from each of the mucus preparations proved to be just as motile as *E. coli* F-18 that had not been in a mouse (Table 3).

Electron microscopy of *E. coli* F-18 grown in L broth and cecal mucus. *E. coli* F-18 cells grown in L broth and in cecal mucus were examined by electron microscopy for the presence of flagella. Of 150 L-broth-grown *E. coli* F-18 cells examined, 30 (20%) had flagella. Similarly, of 98 cecal mucus-grown *E. coli* F-18 cells examined, 18 (18%) had flagella. Since all L-broth-grown cells are motile when

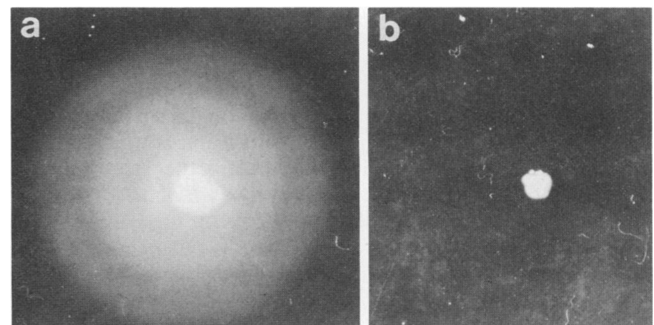

FIG. 2. Spread of *E. coli* F-18 on L motility agar (a) and cecal mucus motility agar (b) after 12 h of incubation at 37°C.

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TABLE 3. Determination of numbers and motility of *E. coli* F-18 isolated from intestinal mucus in vivo[a]

Intestine section	CFU (10^5) per section (mean ± SE)	Diam of spread (mean mm ± SE)[b]
Jejunum	3.5 ± 2.2	24.9 ± 2.8
Cecum	56.7 ± 16.6	21.1 ± 3.1
Proximal colon	58.2 ± 28.7	26.4 ± 2.3
Midcolon	26.6 ± 18.7	29.8 ± 3.1
Distal colon	15.9 ± 7.0	21.6 ± 2.1

[a] Mucus preparations were isolated from three mice colonized with *E. coli* F-18.

[b] Three colonies from each section of each mouse were tested for spread on L motility agar. The diameter of spread of *E. coli* Fla$^-$ in this experiment was 7.2 ± 0.3 mm.

viewed by phase-contrast microscopy, it appears likely that preparation of specimens for electron microscopy shears flagella from *E. coli* F-18. In any case, these data suggest that the cecal mucus-grown *E. coli* F-18 cells were indeed flagellated.

DISCUSSION

We have previously used the streptomycin-treated mouse as a model for large intestine colonization of nonpathogenic gram-negative bacteria and have shown it to be a sensitive tool. For example, lipopolysaccharide-defective mutants of an avirulent *S. typhimurium* strain will not colonize when fed to streptomycin-treated mice together with their smooth parent, although they colonize at normal levels (i.e., about 5 $\times$ 10^7 CFU/g of feces) when fed to mice alone (12, 15). Furthermore, we have shown that a derivative of *E. coli* F-18, *E. coli* F-18 Col$^-$, cannot grow in mouse cecal mucus in vitro in the presence of its parent, although it grows well when inoculated alone and cannot colonize the streptomycin-treated mouse when fed together with its parent, but colonizes in normal numbers when fed to mice alone (6, 19). Moreover, we have shown that *E. coli* F-18 settles to the bottom of culture tubes when grown in nutrient broth containing glucose (1%, wt/wt) and that a nonsettling mutant, *E. coli* F-18 Set$^-$, is a poor colonizer of the streptomycin-treated mouse large intestine when fed to mice together with its parent but not when fed to mice alone (5). These results suggest that the streptomycin-treated mouse large intestine can be used to determine whether a specific defect either in *E. coli* F-18 or in avirulent *S. typhimurium* strains renders them poor colonizers or has no effect on their colonizing abilities and therefore whether the wild-type bacterial component involved is or is not a major colonization factor. It would, of course, be better if we could avoid the use of streptomycin; however, in this case the presence of the normal facultative flora prevents colonization of incoming microorganisms (2, 7, 18).

In this investigation, we have shown that motility/chemotaxis ability does not appear to be a colonization factor for *E. coli* F-18. That is, both a nonflagellated and a nonchemotactic derivative of *E. coli* F-18 colonized the streptomycin-treated mouse large intestine as well as *E. coli* F-18 when either strain was fed to mice together with *E. coli* F-18 (Fig. 1 and Table 1).

We have previously shown that the ability of *E. coli* F-18 to colonize appears to depend on its ability to grow in large intestine mucus (19), and here we have shown that as *E. coli* F-18 grows in large intestine mucus in vitro, it becomes nonmotile (Fig. 2 and Table 2). If the same is true in vivo, i.e., growth in mucus renders *E. coli* F-18 nonmotile, the reason that motility/chemotaxis plays no role in *E. coli* F-18 colonization is clear. Unfortunately, it is difficult to ascertain whether *E. coli* F-18 is nonmotile in mucus in vivo, since it is an extremely small part of the total large intestine mucus flora in streptomycin-treated mice. It should be noted, however, that microscopic examination of the large intestine mucus flora in streptomycin-treated mice reveals that many of the microorganisms are motile and many are nonmotile (unpublished observations).

Flagellar synthesis in *E. coli* is catabolite repressible and as such is relieved by the addition of cAMP (13). In the study presented here, addition of 2 mM cAMP did not cause increased *E. coli* F-18 spread on cecal or colonic mucus motility agar plates (Table 2), suggesting that glucose or glucoselike components present in large intestine mucus were not inhibiting *E. coli* F-18 flagellar synthesis. Indeed, as determined by electron microscopy, *E. coli* F-18 grown in cecal mucus was flagellated, suggesting the possibility that mucus viscosity limited *E. coli* F-18 movement. However, this also appears not to be the case, since actively motile *E. coli* F-18 grown in L broth remained fully motile when suspended in either fresh or spent cecal mucus, and *E. coli* F-18 grown in cecal mucus remained nonmotile when suspended in L broth. Therefore, when grown in cecal mucus, *E. coli* F-18 acts as if it has paralyzed flagella, a phenotype previously described (13).

Finally, we do not know how *E. coli* F-18 travels through mucus in vivo in a nonmotile state to initiate the colonization process. It is possible, however, that as water is resorbed in the intestine to form the fecal pellets found in the proximal colon, *E. coli* F-18 Fla$^-$ or CheA$^-$ cells are passively taken into the mucus layer, where they can then replicate and colonize.

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