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Expression of *Escherichia coli* F-18 Type 1 Fimbriae in the Streptomycin-Treated Mouse Large Intestine

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***Escherichia coli* F-18, isolated from the feces of a healthy human, makes type 1 fimbriae and is an excellent colonizer of the streptomycin-treated mouse large intestine. Recently, it was shown that the inability to produce type 1 fimbriae had no effect on the ability of *E. coli* F-18 to colonize the streptomycin-treated mouse large intestine, suggesting the possibility that *E. coli* F-18 does not express type 1 fimbriae in vivo. However, we show here that *E. coli* F-18 does express type 1 fimbriae in mouse cecal mucus in vivo and, in fact, appears to express substantially more type 1 fimbriae in cecal mucus in vivo than in L broth in vitro.**

Mannose-sensitive binding of *Escherichia coli* bearing type 1 fimbriae to intestinal epithelial cells has long been recognized (4, 5); however, the role of type 1 fimbriae in intestinal colonization has been controversial. Studies of neonatal diarrhea in pigs have shown the infectivity of enterotoxigenic *E. coli* with the type 1 fimbriated phenotype to be several times higher than that of the nonfimbriated counterpart (9), yet other studies have shown that type 1 fimbriae are not produced by enterotoxigenic *E. coli* in the pig small intestine during disease (10). In addition, it has been recently reported that the loss of type 1 fimbriae in a systemically invasive *E. coli* K1 strain did not alter its ability to colonize the large intestines of neonatal rats but did result in a drastic decrease in oropharyngeal colonization (1).

E. coli F-18, which was isolated from the feces of a healthy human, is an excellent colonizer of the streptomycin-treated mouse large intestine (2, 3). *E. coli* F-18 harbors the *fim* operon and makes type 1 fimbriae (8). Furthermore, adhesion of *E. coli* F-18 to specific glycoprotein receptors present in cecal and colonic mucus and to cecal and colonic brush border membranes can be inhibited by D-mannose (12), suggesting that *E. coli* F-18 type 1 fimbriae may mediate adhesion to intestinal mucosal components.

Recently, we reported that *E. coli* F-18 and *E. coli* F-18 FimA$^-$ (an isogenic strain which is unable to make type 1 fimbriae) could, when fed to mice simultaneously, colonize the streptomycin-treated mouse large intestine equally well, each strain persisting at approximately 10^7 CFU per g of feces (8). These data provided clear evidence that the ability to synthesize type 1 fimbriae was not a prerequisite for large intestine colonization and suggested the possibility that *E. coli* F-18 did not produce type 1 fimbriae in the large intestine in vivo.

To test this hypothesis, cecal mucus was collected from six streptomycin-treated mice colonized for 10 days with *E. coli* F-18 and from six streptomycin-treated mice colonized for 10 days with *E. coli* F-18 FimA$^-$. Each sample was placed in 5 ml of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-Hanks buffer (pH 7.4), as described previously (11). Each of the two samples was homogenized in a Dounce homogenizer for 2 min. Forty-five milliliters of HEPES-Hanks buffer (pH 7.4) was then added to each suspension. Each suspension was then left to settle for 20 min at 4°C. The top 45 ml of each suspension was withdrawn and passed through cheesecloth to remove solid debris. The suspensions were then centrifuged for 10 min (5,000 × *g*), and the pellets were resuspended in 5 ml of HEPES-Hanks buffer (pH 7.4). CFU per ml in each suspension was determined by plating on selective medium. Fimbriae were sheared from the bacteria in each suspension by blending five times for 1 min with 1-min intervals on ice. Bacteria were then pelleted by centrifugation for 10 min at 7,710 × *g*, leaving fimbriae in the supernatant. Urea was added to a final concentration of 1 M, and each supernatant was then overlaid on a 1 M urea–5 mM Tris–1 M sucrose cushion buffer and subjected to ultracentrifugation (150,000 × *g*, overnight) as described previously (6). The pellets were resuspended in water and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, as described by Laemmli (7). Proteins separated on the gel were transferred to nitrocellulose and analyzed by Western blotting (immunoblotting) as described by Krogfelt and Klemm (6). Free binding sites on the nitrocellulose were blocked with 2% Tween 20, and the nitrocellulose was incubated with specific anti-type 1 antiserum. Binding of anti-type 1 antibodies to the fimbrial proteins was visualized by subsequent incubation with peroxidase-labeled goat anti-rabbit immunoglobulin G and tetramethylbenzidine. The anti-type 1 antibodies utilized were raised in rabbits against purified type 1 fimbriae by Krogfelt and Klemm (6). Prior to utilization, the antiserum was adsorbed with whole *E. coli* F-18 FimA$^-$ in order to remove nonspecifically reactive antibodies.

Type 1 fimbrial subunits were observed in the cecal mucus of mice colonized by *E. coli* F-18 but not in mice colonized by *E. coli* F-18 FimA$^-$ (Fig. 1, lanes 3 and 4). Interestingly, *E. coli* F-18 appeared to overexpress type 1 fimbriae in mouse cecal mucus in vivo relative to the amount expressed in vitro in L broth (Fig. 1, lanes 2 and 3). That is, judging from the immunoblot signal, 10^7 CFU of *E. coli* F-18 obtained from cecal mucus in vivo appeared to produce significantly more type 1 fimbrial subunits than 10^7 CFU of *E. coli* F-18 grown standing in L broth at 37°C for 48 h and added to cecal mucus. Also, during the type 1 fimbriae purification procedure, 17-kDa (monomer) and 34-kDa (dimer) fimbrial subunits were copurified, yet for reasons which

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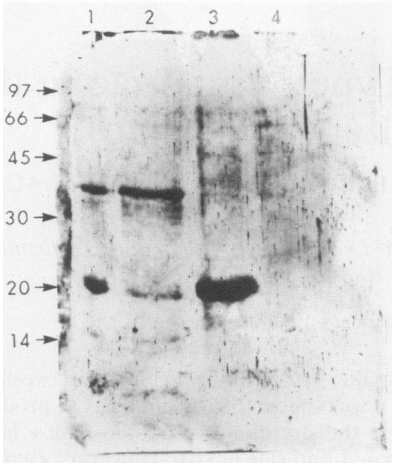

FIG. 1. Western blot analysis of type 1 fimbriae from *E. coli* F-18 grown in vitro and in vivo. Lane 1, purified type 1 fimbriae, 1.5 μg of protein; lane 2, type 1 fimbriae purified from 10^7 CFU of L-broth-grown *E. coli* F-18 added to mucus from mice colonized by *E. coli* F-18 FimA$^-$; lane 3, type 1 fimbriae from 10^7 CFU of *E. coli* F-18 purified directly from mucus of mice colonized by *E. coli* F-18; lane 4, same purification procedure as in lane 3, but from mucus of mice colonized by *E. coli* F-18 FimA$^-$. The positions of protein molecular mass standards (in kilodaltons) are indicated on the left.

are unclear, the dimer form appears to predominate when *E. coli* F-18 is grown in L broth and added to cecal mucus (Fig. 1, lane 2), whereas the monomer form appears to predominate when type 1 fimbriae are prepared from *E. coli* F-18 which have colonized cecal mucus (Fig. 1, lane 3).

In summary, despite the fact that type 1 fimbriae are not necessary for *E. coli* F-18 to colonize the streptomycin-treated mouse large intestine (8), *E. coli* F-18 does express substantial amounts of type 1 fimbriae in mouse cecal mucus in vivo.

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