Altered Colonizing Ability for Mouse Large Intestine of a Surface Mutant of a Human Faecal Isolate of *Escherichia coli*

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*Escherichia coli* F-17 Sr, a human faecal isolate, is resistant to the T-series of bacteriophages (i.e. T2 to T7). A T2-sensitive mutant of *E. coli* F-17 Sr was isolated following acriflavin treatment. This mutant, *E. coli* F-17 Sr Ts was found to be sensitive to the entire T-series of phages. *E. coli* F-17 Sr and *E. coli* F-17 Sr T5 did not differ quantitatively in total LPS content. However, analysis of LPS revealed that a large fraction of *E. coli* F-17 Sr Ts was devoid of O-side-chains. This accounted for the sensitivity of this strain to bacteriophages T3, T4, and T7. In addition, *E. coli* F-17 Sr Ts contained only about half the amount of capsular material contained by *E. coli* F-17 Sr accounting for the sensitivity of the mutant to bacteriophages T2, T5, and T6. Although the two strains colonized equally well when fed individually to streptomycin-treated mice, when fed simultaneously to streptomycin-treated mice, *E. coli* F-17 Sr Ts colonized at a level of about 1 \times 10^8 cells (g faeces)^{-1}, whereas *E. coli* F-17 Sr colonized at only 1 \times 10^4 cells (g faeces)^{-1}. These studies suggest that bacterial cell surface components modulate the large intestine colonizing ability of *E. coli* F-17 Sr in the mouse large intestine.

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**INTRODUCTION**

Although *E. coli* is a normal inhabitant of the human intestinal tract, pathogenic strains can cause traveller's diarrhoea, infant diarrhoea or neonatal colibacillosis of piglets and calves (Bray, 1945; Taylor *et al.*, 1949; Ørskov and Ørskov, 1966; Formal *et al.*, 1971; Ørskov *et al.*, 1975). Smith & Linggood (1971) demonstrated that these enterotoxigenic *E. coli* (ETEC), which exert their pathogenic effect in the proximal small intestine, must have accessory virulence properties in addition to enterotoxin in order to cause disease in piglets. This has also been clearly demonstrated in humans (DuPont *et al.*, 1971). The emerging picture is that the pathogenesis of ETEC strains is mediated through plasmids which determine adhesion to intestinal mucosa, as well as enterotoxin production (Evans *et al.*, 1975; Burrows *et al.*, 1976; Nagy *et al.*, 1977; Evans & Evans, 1978; Gyles *et al.*, 1978; Clancy & Savage, 1981).

In contrast to the situation regarding enterotoxigenic strains of *E. coli*, relatively little information is available concerning the basis for colonization of the large intestine by normal faecal *E. coli* strains. However, microscopic evidence suggests that *E. coli* adheres to the mucus-secreting brush border cells of human colon epithelium (Hartley *et al.*, 1979).

In a previous report, we were able to classify *E. coli* strains with respect to their ability to colonize the large intestine in streptomycin-treated CD-1 mice (Myhal *et al.*, 1982). For example, when *E. coli* K12 or a human faecal *E. coli* strain were individually fed to streptomycin-treated mice, each strain persisted indefinitely at a concentration of about 1 \times 10^8 cells (g faeces)^{-1}. However, when *E. coli* K12 organisms were fed to streptomycin-treated mice simultaneously with the human faecal strain, it retained, at most, 1% of its individual colonizing ability. In contrast, the human faecal strain retained all of its individual colonizing ability.

Abbreviations: ETEC, enterotoxigenic *Escherichia coli*; KDO, 2-keto-3-deoxyoctonate.
Because of the importance of surface adherence factors to colonization by ETEC in the small intestine, it became of interest to alter the surface of an E. coli strain recently isolated from human faeces and to determine whether such an alteration could be correlated with a change in colonizing ability in the mouse large intestine. The results of this investigation show that an alteration in the cell surface of an E. coli strain does indeed affect its colonizing ability.

METHODS

Bacteria and bacteriophage. Escherichia coli F-17 was isolated in 1977 from the faeces of a healthy student from University of Rhode Island by the method of Dufour et al. (1981). Escherichia coli strain J5-3, a K12 strain, was obtained from Dr G. M. Thorne, Tufts Medical School, Boston, Mass., U.S.A. Escherichia coli K12 (K88ab) contains the K88ab plasmid and was obtained from Dr R. Wilson, E. coli Reference Center, The Pennsylvania State University, University Park, Pa., U.S.A. Escherichia coli B was from the collection of Dr Paul S. Cohen. Spontaneous streptomycin-resistant (Sr), or rifampicin-resistant (Rif) mutants were selected by plating between 1 × 10⁸ and 1 × 10⁹ cells on L agar plates containing the appropriate antibiotic. Resistance is designated with a superscript r and sensitivity with a superscript s. Selective markers of the strains used in the experiments presented here are described in the legends to the figures. Bacteriophages T2, T3, T4, T5, T6, and T7 were from the collection of Dr Paul S. Cohen.

Mouse colonization experiments. Male CD-1 mice (35-42 d old; Charles River Mouse Farms) were given drinking water containing streptomycin sulphate [5 g (1 sterilized water)-¹] for 1 d to clear their intestines of streptomycin-sensitive bacteria. After 1 d of streptomycin treatment, the count of facultative bacteria dropped from approximately 1 × 10⁸ organisms (g faeces)⁻¹ to an undetectable number on L agar plates (i.e. less than 10² organisms (g faeces)⁻¹). Mice were then starved overnight and the next morning groups of six animals were fed with approximately 1 × 10⁹ organisms of each of the E. coli strains to be tested. Each mouse ate bacteria mixed with 1 ml sterile 20% (w/v) sucrose and contained in test tube caps. After feeding, the animals were returned to their normal diet (Charles River Valley Rat, Mouse, Hamster Formula) including sterile drinking water containing streptomycin. At 24 h intervals thereafter, 1 g faeces was collected from each mouse; this was homogenized in 10 ml of L broth, diluted and plated on the appropriate selective media. In this investigation, colonizing ability was assessed by the level at which a strain persisted in mouse faeces.

Coliphage biotyping. A strain to be tested (approximately 2 10⁸ organisms ml⁻¹) was mixed in 3 ml L broth containing 0-7% (w/v) agar (46 °C) and poured over the surface of an L agar plate. Approximately 20 min later, when the agar overlay had hardened, specific coliphages (about 1 10⁶ p.f.u.) were spotted on to designated areas on the plate with an inoculating loop. The plates were then incubated at 37 °C for 24 h prior to scoring.

Bacteriophage adsorption. Adsorptions were all performed in L broth using approximately 2 10⁸ exponential phase E. coli organisms ml⁻¹ and approximately 1 10⁶ p.f.u. Incubations were at 37 °C for 5 min, following which the centrifuged tissue for 10 min at 5000 g and at 4 °C to sediment adsorbed bacteriophage. Supernatants were titrated on E. coli B using the soft agar method of Adams (1959). Adsorptions were essentially complete in 5 min.

Isolation of E. coli F-17 Sr Ts. A standing culture of E. coli F-17 Sr Ts was grown overnight in L broth containing 5 μg acriflavin ml⁻¹ at 37 °C to a density of about 2 10⁸ organisms ml⁻¹. L agar plates were then spread with about 100 acriflavin-grown F-17 Sr Ts cells each and incubated at 37 °C for 18 h. Individual colonies were tested for their sensitivity to bacteriophage T2. One of 2600 colonies was found to be a T2-sensitive mutant and was designated F-17 Sr Ts.

Isolation and characterization of plasmid DNA. The method of Guerry et al. (1973) as modified by Meyers et al. (1976) was used to purify plasmid DNA. Following plasmid DNA purification, the presence of specific plasmids was determined by agarose slab gel electrophoresis as described by Meyers et al. (1976).

2-Keto-3-deoxyoctonate (KDO) measurements. KDO measurements were made on whole E. coli cells and on purified LPS preparations were made by the method of Karkhanis et al. (1978).

Hexose measurements. Hexose was measured using the indole method (Dische, 1955) with galactose as the standard.

Protein measurements. Escherichia coli protein was measured by the Lowry method, with bovine albumin as the standard.

LPS purification. LPS was purified from E. coli by the warm phenol/H₂O method of Westphal & Jann (1965).

PAGE of E. coli LPS. Purified E. coli LPS preparations were electrophoresed and silver stained on 14% (w/v) SDS-polyacrylamide gels as described by Tsai & Frasch (1982).

Capsule weights. Capsule was purified from the supernatant obtained after sedimentation of E. coli LPS from the aqueous phase of a warm H₂O/phenol extract of one litre of E. coli cells. The aqueous phase was lyophilized and the capsular polysaccharide obtained was weighed using a Mettler HE10 balance equipped with a Mettler BA28 digital readout. Capsule preparations contained less than 10% nucleic acid and less than 1% protein.
E. coli colonization

Hydrophobic-interaction chromatography. Retention of bacteria on phenyl-Sepharose columns was measured as described by Jann et al. (1981).

Isolation and characterization of outer membrane proteins. Outer membranes of E. coli strains F-17 S', F-17 S' T', and J5-3 were isolated by the method of Schnaitman (1974). Following isolation, outer membranes were methylated using [3H]formaldehyde [2 μmol (mg protein)⁻¹, 65 μCi (2.4 MBq) μmol⁻¹] by the method of Jentoft & Dearborn (1979). Tritium-labelled proteins were analysed on 10% (w/v) polyacrylamide slab gels as described previously (Lynch et al., 1979). Dried gels were fluorographed according to the procedure of Laskey & Mills (1975).

Media, antibiotics and plating efficiencies. L. broth was made as described by Revel (1967) and L agar is L broth containing 12 g Bacto Agar l⁻¹. Davis Minimal Broth (Difco) was supplemented with dextrose (1 g 1⁻¹). Streptomycin sulphate and rifampicin, were purchased from Sigma. Antibiotic-resistant mutants all had equal plating efficiencies on L agar and L agar containing the antibiotics to which they are resistant (100 μg streptomycin sulphate ml⁻¹ and 50 μg rifampicin ml⁻¹).

Chemicals. [3H]Formaldehyde (65 mCi mmol; 2.4 GBq mmol⁻¹) was from New England Nuclear, Boston, Mass., U.S.A. Phenyl-Sepharose CL-4B and 2-keto-3-deoxyoctonate were from Sigma. All other chemicals were reagent grade.

RESULTS

Bacteriophage sensitivity and colonizing ability of E. coli F-17 S'

Escherichia coli F-17 S' was resistant to bacteriophages T2, T3, T4, T5, T6, and T7 by the coliphage biotyping test (see Methods). In addition, adsorption studies showed that E. coli F-17 S' adsorbed the bacteriophages very poorly (Table 1). When E. coli F-17 S' was fed to mice it colonized indefinitely at a level equal to that of other normal human faecal strains (i.e., approximately 1 x 10⁸ organisms (g faeces)⁻¹ (Myhal et al., 1982)). The rif² mutant of E. coli F-17 S' also colonized at this level when fed to mice, alone or together with its S' parent (Fig. 1a) and was used in subsequent colonization experiments.

Bacteriophage sensitivity and colonizing ability of E. coli S' T'

Escherichia coli F-17 S' T' was isolated as a T2-sensitive mutant of E. coli F-17 S' (see Methods). Escherichia coli F-17 S' T' was sensitive to (as assessed by coliphage biotyping) the remaining members of the T-series of bacteriophages especially T2, T6, and T7 and the mutant also adsorbed these phages (Table 1). When fed to mice together with E. coli F-17 S' rif², E. coli F-17 S' T' colonized at about 1 x 10⁸ organisms (g faeces)⁻¹, whereas E. coli F-17 S' rif² colonized at a level approximately four orders of magnitude lower (Fig. 1b). In this test system, therefore, the bacteriophage sensitive mutant colonized to a level 10⁴-fold greater than the parent strain (Fig. 1b).

Plasmid profiles

Acriflavin, which was used in isolating E. coli F-17 S' T', is a plasmid curing agent (Mitsuhashi et al., 1961). However, plasmid profiles of F-17 S' and F-17 S' T' were identical (Fig. 2), showing that plasmid loss was not involved in the change from bacteriophage resistance to sensitivity.

Growth studies

Standing cultures of E. coli F-17 S' and E. coli F-17 S' T' were found to have identical generation times of 30 min when grown either together or individually at 37°C in Davis Minimal Broth containing glucose as the carbon source (data not shown). This result showed that the mutation of F-17 S' to T-phage sensitivity did not alter its simple nutritional requirements and that neither strain killed the other in mixed culture. It is therefore unlikely that the different colonizing abilities of the two strains are due to colicins or to metabolic differences.
Table 1. Bacteriophage adsorption to E. coli F-17 S' and E. coli F-17 S' T*

Adsorptions were performed for 5 min at 37°C as described in Methods.

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-17 S'</td>
<td>82</td>
<td>77</td>
<td>96</td>
<td>76</td>
<td>86</td>
<td>81</td>
</tr>
<tr>
<td>F-17 S' T*</td>
<td>1.2</td>
<td>15</td>
<td>48</td>
<td>28</td>
<td>6.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Fig. 1. Relative colonizing abilities of E. coli F-17 strains in the mouse intestine. (a) E. coli F-17 S' and E. coli F-17 S' rif were fed simultaneously to streptomycin-treated mice as described in Methods. Samples were plated on L agar containing 100 μg streptomycin sulphate ml⁻¹ and 50 μg rifampicin ml⁻¹. The number of E. coli F-17 S' organisms (g faeces)⁻¹ was determined by subtracting the number of E. coli F-17 S' rif organisms (g faeces)⁻¹ (determined by counts on plates containing both streptomycin and rifampicin) from the total number of E. coli organisms (g faeces)⁻¹ (determined by counts on plates containing streptomycin). Counts (mean values ± s.e. from each of six animals are expressed as log₁₀ [no. organisms (g faeces)⁻¹]. ○, E. coli F-17 S'; ●, E. coli F-17 S' rif. (b) Mice were fed E. coli F-17 S' rif (●) and E. coli F-17 S' T* (△), and assayed as in (a).

Surface properties of E. coli F-17 S' and E. coli F-17 S' T*

Amount of KDO (mg bacterial cell protein)⁻¹. Escherichia coli LPS contains three residues of KDO per oligosaccharide chain of LPS (Nikaido, 1973). The amount of KDO (mg bacterial cell protein)⁻¹ therefore is in proportion to the amount of LPS present. By this criterion, E. coli F-17 S' and E. coli F-17 S' T* had the same amount of LPS per cell (Table 2).

Hexose : KDO ratio. A comparison of the ratio of hexose to KDO in E. coli F-17 S' LPS to that of E. coli F-17 S' T* LPS was taken as a reflection of the relative average lengths of the O-side-chains of the two strains. As shown in Table 3, the hexose to KDO ratio of the LPS of E. coli F-17 S' was almost twice that of E. coli F-17 S' T*, suggesting that the average O-side-chain length of E. coli F-17 S' T* LPS was significantly less than that of E. coli F-17 S' LPS.

SDS-PAGE of E. coli F-17 S' and E. coli F-17 S' T* LPS. It has recently been shown by PAGE that LPS purified from an E. coli strain is not homogeneous with respect to O-side-chain length (Goldman & Leive, 1980; Palva & Makela, 1980). In fact, as many as 40 LPS components of different molecular weights have been observed in some cases (Goldman & Leive, 1980; Palva & Makela, 1980). The two lowest molecular weight LPS bands have been shown to consist of lipid A and core oligosaccharide devoid of O-side-chains and are called R type molecules. Each LPS component in ascending order is presumably increased in molecular weight by one tetrasaccharide O-side-chain repeating unit (Goldman & Leive, 1980; Palva & Makela, 1980).
Fig. 2. Plasmid profiles of *E. coli* F-17 S' and *E. coli* F-17 S' Tβ analysed on agarose gels. A, *E. coli* F-17 S'; B, *E. coli* F-17 S' Tβ. The arrow indicates *E. coli* chromosomal fragments. The remaining bands represent plasmid DNA.

**Table 2. KDO content of *E. coli* parent and mutant strains**

Cultures (100 ml) were assayed for total protein and for KDO (see Methods).

<table>
<thead>
<tr>
<th><em>E. coli</em> strain</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-17 S'</td>
<td>3.8*</td>
<td>3.4</td>
</tr>
<tr>
<td>F-17 S' Tβ</td>
<td>3.8</td>
<td>3.9</td>
</tr>
</tbody>
</table>

* This culture contained 65.0 μg KDO and 17.2 mg cell protein. All cultures were similar in protein content.
Table 3. Ratio of hexose to KDO in LPS extracted from E. coli parent and mutant strains

Hexose and KDO were measured as described in Methods.

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-17'</td>
<td>10.6</td>
<td>11.2</td>
</tr>
<tr>
<td>F-17' T'</td>
<td>5.9</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Fig. 3. SDS-PAGE of E. coli F-17 S' and E. coli F-17 S' T' purified LPS preparations. Purified LPS preparations were made, electrophoresed, and stained as described in Methods. Lanes 1 and 4, E. coli J5-3 LPS; lanes 2 and 5, E. coli F-17 S' T' LPS; lanes 3 and 6, E. coli F-17 S' LPS. Lanes 1, 2, and 3 contained 0.5 µg LPS and lanes 4, 5, and 6 contained 1.0 µg LPS with respect to KDO. R indicates R-type LPS.

PAGE was performed on E. coli F-17 S' and F-17 S' T' purified LPS preparations. Escherichia coli F-17 S' LPS contained significant amounts of both high and low molecular weight LPS molecules (Fig. 3). Relatively few R type LPS molecules were observed. In contrast, E. coli F-17 S' T' LPS contained significant amounts of both R type and high molecular weight LPS molecules, but relatively few lower molecular weight LPS molecules (Fig. 3). Escherichia coli J5-3, a K12 strain which only contains R type LPS, is also shown in Fig. 3 as a control. The fact that F-17 S' T' has large amounts of R type LPS probably accounts for the sensitivity of this strain to bacteriophages T3, T4, and T7, since only R type LPS molecules adsorb these bacteriophages (Lindberg, 1973).

Capsular material. Capsule dry weight, expressed as mg dry wt (mg protein)^-1, was taken as a reflection of the amount of capsular material. Escherichia coli F-17 S' contained approximately twice as much capsule as E. coli F-17 S' T' (Table 4). This finding may explain the sensitivity of F-17 S' T' and the resistance of F-17 S' to bacteriophages T2, T5, and T6. These bacteriophages adsorb to the E. coli outer membrane (Braun & Hantke, 1981) and it is possible that the large amount of capsule on E. coli F-17 S' prevents their adsorption.
Table 4. Capsular content of E. coli parent and mutant strains

Dry weight determinations were made on capsule extracted from cultures (1 1) as described in Methods.

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-17 S'</td>
<td>0.131*</td>
<td>0.128</td>
</tr>
<tr>
<td>F-17 S'T'</td>
<td>0.071</td>
<td>0.064</td>
</tr>
</tbody>
</table>

* In this case, the E. coli F-17 S' culture contained 26.8 mg capsule and 204 mg protein.

Fig. 4. Outer membrane proteins of E. coli F-17 S' and E. coli F-17 S'T'. Outer membrane proteins were prepared, labelled, and analysed (100000 c.p.m. of each preparation) as described in Methods. Lane 1, E. coli F-17 S'; lane 2, E. coli S'T'; lane 3, E. coli J5-3. Molecular weights (10^{-3} \times \text{Dal}) of specific E. coli J5-3 proteins are indicated by arrows.
Surface hydrophobicity. It is thought that the surface hydrophobicity of an E. coli strain plays a role in its ability to adhere to eukaryotic cells and therefore in its colonizing ability (Smyth et al., 1978). We therefore determined relative surface hydrophobicities of E. coli strains F-17 S' and F-17 S' T' by hydrophobic interaction chromatography. The extent of hydrophobicity is assessed by retention on phenyl-Sepharose (Jann et al., 1981; Smyth et al., 1978). In two experiments, the average percentage retention on phenyl-Sepharose of F-17 S' and F-17 S' T' was 50 and 42, respectively. In contrast, E. coli K12 (K88ab), a positive control (Jann et al., 1981), was retained to the extent of 76% and E. coli J5-3, a negative control, to the extent of 25%. It therefore appears that E. coli F-17 S' and E. coli F-17 S' T' are similar in this respect, midway between very high and very low surface hydrophobicities.

Outer membrane proteins. The outer membrane proteins of E. coli F-17 S' and E. coli F-17 S' T' were compared by PAGE (see Methods). The outer membrane protein profiles of the two strains were very similar (Fig. 4). In some experiments, E. coli F-17 S' T' contained considerably less of a 18000 Dal protein, but this difference was not consistent (Fig. 4). The outer membrane proteins of E. coli J5-3, a K12 strain, are also shown in Fig. 4, as a reference.

DISCUSSION

In the present investigation, we have observed two major differences in the surfaces of E. coli F-17 S' and E. coli F-17 S' T'. Firstly, E. coli F-17 S' had about twice as much capsular material as E. coli F-17 S' T'. Secondly, the mutant differed markedly from the parent strain in content of LPS O-specific side-chains. Escherichia coli F-17 S' T' lacked many of the lower molecular weight side-chains that were present in F-17 S'. However, E. coli F-17 S' T' contained many R type LPS molecules, which were not present on E. coli F-17 S'.

Pleiotropic membrane effects have been observed previously among E. coli LPS mutants. For example, E. coli K12 strains, resistant to bacteriophages T3, T4, and T7 have been isolated and shown to be LPS heptose-deficient mutants whose outer membrane protein composition has also been affected (Havekes et al., 1976).

The extent to which observed surface differences are involved in the increased mouse colonization ability of E. coli F-17 S' T' relative to E. coli F-17 S' is at present unknown. It should be mentioned, however, that LPS has recently been implicated in the adhesion of a nonpiliated clinical strain of Shigella flexneri 1b to guinea pig colonic epithelium (Izhar et al., 1982). Clearly, more research is needed to identify the relative importance of LPS and capsule in the E. coli colonization of the large intestine.

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REFERENCES


