Expression of cellulose and curli fimbriae by
Escherichia coli isolated from the gastrointestinal tract

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Escherichia coli colonizes the gastrointestinal tract of humans; however, little is known about the features of commensal strains. This study investigated whether expression of the biofilm extracellular matrix components cellulose and curli fimbriae is found among commensal isolates. Fifty-two E. coli strains were isolated from faecal samples and, as a control, 24 strains from urinary tract infections were also used. Faecal isolates were characterized by serotyping and phylogenetically grouped by PCR. The genotype was determined by PFGE and the presence of virulence factors was assessed. Co-expression of cellulose and curli fimbriae at 28 °C and 37 °C was typical for faecal isolates, while urinary tract infection strains typically expressed the extracellular matrix components at 28 °C only. Knockout studies in a representative faecal isolate revealed that the response regulator CsgD regulated cellulose and curli fimbriae, as found previously in Salmonella enterica. In contrast to S. enterica, at 37 °C pellicle formation occurred in the absence of cellulose and curli fimbriae. The gastrointestinal tract represents a source of biofilm-forming bacteria, which can spread to susceptible sites.

INTRODUCTION

Escherichia coli is a component of the natural gastrointestinal flora of almost any human. It is one of the first colonizers of the gastrointestinal tract of newborns, being either transferred from the mother during delivery or acquired by environmental sources (Bettelheim et al., 1974). After establishment of a stable microbial population, E. coli is, with 1 % of the total bacterial biomass, a minor component of the commensal flora; however, with up to 10^8 cells ml^-1 it reaches significantly high concentrations (Hartl & Dykhuizen, 1984; Leclerc et al., 2001). These high absolute numbers make the gastrointestinal tract a major source for the spread of potentially pathogenic E. coli to susceptible sites via the faecal route (Brauner et al., 1991; Johnson et al., 1998). Translocation of enteric bacteria, in particular E. coli, over the gut epithelial cell lining can cause sepsis in susceptible individuals (Berg, 1999; Guarner & Malagelada, 2003; O’Boyle et al., 1998). Furthermore, predominance of E. coli in early and chronic ileal lesions of Crohn’s disease (Chadwick & Chen, 1999; Darfeuille-Michaud et al., 1998), an inflammatory bowel disease, shows the versatility of the pathogenic potential displayed by this organism.

The commensal E. coli population was reported to be distinct from isolates with pathogenic potential as it displays a lower frequency of virulence traits such as adherence factors and toxins (Cooke & Ewins, 1975). However, some adhesins like P fimbriae play a dual role, since they enhance the colonization capacity in the intestine as well as enable the cells to colonize the urogenital tract and cause pyelonephritis (Wold et al., 1992). Type 1 fimbriae have no role in gut colonization (Bloch & Orndorff, 1990; McCormick et al., 1989), but adherence and invasion of intestinal epithelial cells mediated by type 1 fimbriae is a feature of E. coli strains isolated from lesions of Crohn’s disease (Boudeau et al., 2001).

The red, dry and rough (rdar) morphotype, a multicellular behaviour, includes adhesion to abiotic surfaces (biofilm formation) and expression of curli fimbriae and cellulose as extracellular matrix components. Curli fimbriae and/or cellulose are expressed by E. coli, Salmonella spp. and other Enterobacteriaceae (Collinson et al., 1991; Hammar et al., 1995; Olsen et al., 1989; Römling et al., 1998a, 2003; Zogaj et al., 2001, 2003). While the contribution of cellulose to virulence has not been further investigated, several

†These two authors contributed equally to this paper.

Abbreviation: UTI, urinary tract infection.
virulence-associated features have been assigned to curli fimbriae (Herwald et al., 1998; Olsen et al., 1989, 2002; Sjöbring et al., 1994). However, a common picture of the role of curli fimbriae in pathogenicity is still elusive, since curli fimbriae are not consistently expressed by \textit{E. coli} pathovars. While the majority of sepsis isolates have been found to express curli fimbriae even at 37°C, enteropathogenic \textit{E. coli} (EPEC), enterotoxigenic \textit{E. coli} (ETEC) and uropathogenic \textit{E. coli} (UPEC) show expression only at ambient temperature. In contrast, enteroinvasive \textit{E. coli} (EIEC), \textit{Shigella} spp. and enterohaemorrhagic \textit{E. coli} (EHEC) regularly do not express curli fimbriae in vitro (Bian et al., 2000; Cookson et al., 2002; Patri et al., 2000; Sakellaris et al., 2000; Sjöbring et al., 1994; Uhlich et al., 2001).

Expression of cellulose requires the \textit{bcs}ABZC operon encoding structural genes for cellulose biosynthesis (Zogaj et al., 2001), with the catalytic subunit of the cellulose synthase encoded by \textit{bcsA}. The biosynthesis of curli fimbriae is performed by two divergently transcribed operons \textit{csg}DEFG and \textit{csg}ABC. Curli fimbriae are composed of CsgA, the structural protein subunit. CsgD, a transcriptional response regulator of the LuxR superfamily, is required for the activation of curli as well as cellulose biosynthesis. This genetic network leading to rdar morphotype expression has mainly been studied in \textit{Salmonella enterica} serovar Typhimurium (S. Typhimurium) ATCC 14028, while regulation of curli expression was also investigated in \textit{E. coli} K-12 strains (Hammar et al., 1995; Römling et al., 1998a, 2000; Zogaj et al., 2001).

The expression of the extracellular matrix components, curli fimbriae and cellulose, has never been investigated in commensal isolates. Therefore, we examined the faecal \textit{E. coli} population of healthy individuals for the expression of curli fimbriae and cellulose, and compared it to a population of strains from urinary tract infections (UTIs). Co-expression of curli fimbriae and cellulose at 28°C and 37°C was typical for faecal isolates, while expression of curli fimbriae and cellulose at 28°C was typical for faecal isolates, while expression of curli fimbriae and cellulose at 28°C only was the predominant morphotype in UTI isolates. Biofilm formation was associated with the expression of the two matrix components. A typical faecal isolate, \textit{E. coli} TOB1, which expressed the rdar morphotype at 28°C and 37°C, was chosen for further investigation. Knockout of the structural genes for cellulose and curli fimbriae confirmed expression of these extracellular matrix components. CsgD positively regulated the expression of cellulose and curli fimbriae, as previously described for \textit{S. Typhimurium} ATCC 14028. In contrast to \textit{S. Typhimurium}, biofilm behaviour was also observed when cellulose and curli fimbriae were not expressed. While biofilm formation by pathogenic and commensal \textit{E. coli} was hypothesized to occur in the intestine (Cassels & Wolf, 1995), we report for the first time biofilm formation of commensal \textit{E. coli} isolates, which might have an impact on the spread of biofilm-forming organisms to susceptible sites as well as on the interaction with gastrointestinal epithelial cells.

### METHODS

#### Study design.

Eleven members of three family households agreed to participate in the study and informed consent was obtained (age range, 0-4 to 45 years; mean age, 22.5 years). All subjects were healthy and collected faecal swabs (at the collector’s discretion). For statistical purposes, faecal swabs were collected from 10 additional individuals. The UTI samples were those received in a routine clinical laboratory during 2 days. No selection was made with respect to the type of UTI infection, but only samples collected were those where obviously pure \textit{E. coli} infections were observed.

#### Ethics.

The local ethical committee approved the study.

#### Microbiological methods.

Since instability of morphotypes was expected, primary bacterial isolates were collected. After collection, individuals were asked to inoculate the faecal swabs immediately on MacConkey agar plates. Plates were incubated overnight at room temperature or at 37°C. Primary urine streaks on blood agar plates or Klinger-Iron plates were collected from the local hospital after overnight growth.

The confluent microbial outgrowth was collected with a sterile loop and vigorously resuspended in PBS. Tenfold dilutions were plated on MacConkey agar plates until single colonies were obtained. After incubation overnight at 37°C, plates were replica-plated onto Congo red (CR) plates [Luria–Bertani (LB) agar plates without salt supplemented with CR]. CR plates were either incubated for 24 h at 37°C or for 48 h at 28°C.

Colony morphology was inspected on MacConkey and CR plates, and two colonies of each unique colony morphology pattern were saved. Colony morphologies on CR plates were scored according to the basic morphotypes previously detected in \textit{S. Typhimurium} (Fig. 1a; Römling et al., 2000); rdar (violet colony, expresses curli fimbriae and cellulose), pdar (pink colony, expresses cellulose), bdar (brown colony, expresses curli fimbriae) and saw (no expression of curli fimbriae nor cellulose). A less-pronounced phenotype was reported as ras (violet and smooth), bas (brown and smooth) or pas (pink and smooth). The frequency of the individual morphotypes was reported on the basis of the examination of at least 100 colonies per sample considering the phenotype on MacConkey as well as CR agar plates. Identification of isolates was done with an API 20E kit (BioMerieux). For subsequent analysis, bacterial strains were usually grown in LB broth without salt or on plates made with the same broth for 24 h at 37°C or 48 h at 28°C.

#### Molecular typing methods.

Two different kinds of molecular typing method were used to characterize the faecal \textit{E. coli} strains. Phylogenetic grouping was performed using a triplex PCR targeting the genes \textit{chuA}, \textit{yjaA} and \textit{TspE4-C2} as described elsewhere (Clermont et al., 2000). With this method, four different phylogenetic groups, A, B1, B2 and D, can be discriminated.

Typing on the level of strains was done using macrorestriction fingerprints resolved by PFGE. High-molecular-mass DNA in agarose plugs was prepared as described previously (Römling et al., 1994). Isolated DNA was digested with the restriction enzyme \textit{XbaI} and plugs were loaded onto a 1.5% agarose gel in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.3). DNA was subjected to separation in a CHEF-Mapper (Bio-Rad) using the following parameters: pulse-times: 1–14 s for 14 h, 12–25 s for 22 h, 8–50 s for 24 h; 6 V cm⁻¹; 120° reorientation angle. Evaluation of macrorestriction fragment patterns was done as described elsewhere (Struelens, 1996). Isolates with an identical pattern were designated the same pulsed-field type, while isolates with up to three fragment differences were defined as subtypes.

#### Detection of virulence factors.

The presence of adhesins and other
virulence traits was performed by PCR using primers as described recently (Novrouzian et al., 2001). The following adhesins were investigated: P fimbriae (presence of papC), S fimbriae (sfaD, sfaE) and Dr haemagglutinin (draA). P fimbriae were further classified according to the class of the PapG adhesin (classes I, II and III). Other virulence traits investigated were: capsule K1 (nuvB), capsule K5 (kfiC), aerobactin (iutA) and haemolysin (hlyA).

**Phenotypic screens.** M9 minimal medium was used to check for auxotrophy. Haemolytic activity was judged after growth on sheep blood agar plates at 37 °C for 24 h.

**Detection of curli fibres.** Since curli fibres tend to aggregate, we developed an enrichment procedure for CsgA before visualization and detection on protein gels and Western blots. Three milligrams of bacteria was harvested from plates and resuspended in 1.5 ml T E buffer used in the separation gel and the running buffer). Visualization of curli fibre subunits was done after colloidal Coomassie staining and detection by Western blotting using an anti-CsgA antibody at a dilution of 1 : 1000.

Alternatively, CsgA subunits were detected by matrix-associated laser desorption-time of flight (MALDI-TOF) analysis (Römling et al., 2003). The CsgA-enriched pellet resuspended in formic acid was applied to (a) Representative morphotypes of E. coli isolates from faeces and UTI infections. As a reference, S. Typhimurium morphotypes saw (MAE51; no cellulose/no curli fimbriae), pdar (MAE97; cellulose/no curli fimbriae), bdar (MAE171; no cellulose/curli fimbriae) and rdar (MAE52; cellulose/curli fimbriae) are shown (indicated with ‘c’). The E. coli strains shown are: 1, Fec75; 2, Fec5; 3, Fec41; 4, Fec69; 5, TOB1. Strains were grown for 24 h at 37 °C. (b) Morphotype distribution in faecal (grey) and UTI (black) isolates. Strains are ordered from left to right according to decreased expression of extracellular matrix components. Letters: r, rdar/ras; b, bdar/bas; p, pdar/pas; s, saw; m, mucoid.

**Biofilm assay.** Biofilm development (Römling, 2001) was observed in microtitre plates without shaking using M9 and LB without salt as incubation media after 24 h at 37 °C and 48 h at 28 °C. Cell clumping and pellicle formation was inspected visually. Adherence was determined by crystal violet staining and quantified after the dye was dissolved in 250 μl 100 % dimethyl sulfoxide for 1.5 h at 37 °C. Scores for cell clumping, pellicle formation (2X) and adherence were added to a common score assessing the biofilm-formation capacity.

**Molecular biology methods.** Molecular biology methods were carried out using standard procedures (Ausubel et al., 1994). One-step knockout of csgD and bcsA was carried out according to the protocol of Datsenko & Wanner (2000), with the exception that up to 2 μg PCR product was electroporated into the target strain to achieve the gene knockout. Primers used were as follows: for csgD encoding the regulatory component of extracellular matrix components, Ec_CsgD_Start (ATGTTTAAATG AAGTCCATAGTATCAGTACATATGTGTTGATCACGTTG TTGAGCATCCGTTGAGCTGCTCTG) and Ec_CsgD_Stop (TTATATCACGTTA GTAT-GTTTGGCCACGAAAAGCCATTGCTGTCCGATTATAC TATCCTCCTTCTGTTGTTATGAAATATATG). For bcsA encoding the catalytic subunit of cellulose synthase, Ec_bcsA_Start (ATGTTTAAATG AAGTCCATAGTATCAGTACATATGTGTTGATCACGTTG TTGAGCATCCGTTGAGCTGCTCTG) and Ec_bcsA_Stop (TTATATCACGTTA GTAT-GTTTGGCCACGAAAAGCCATTGCTGTCCGATTATAC TATCCTCCTTCTGTTGTTATGAAATATATG). Gene deletions were confirmed by PCR.

Expression of csgD was induced by addition of 0.1 % arabinose to the medium. Constructed mutants are listed in Table 1.
Table 2. Classification of isolates on the level of individual phylogenetic groups rarely harboured virulence factors. The same phenomenon was found (Table 2). Strains from several virulence factors (Duriez et al., 2001). Earlier studies showed that commensal and virulent strains are gathered in group B2 and that potentially virulent strains of group B2 had gathered several virulence factors (Duriez et al., 2001). In this study, the same phenomenon was found (Table 2). Strains from other phylogenetic groups rarely harboured virulence factors (Table 2). Classification of isolates on the level of individual strains by PFGE revealed a high diversity of isolates, while closely related strains (clonal variants) were shared among family members (Fig. 2, Table 2). Clonal variants could display different morphotypes even within an individual.

Table 1. Strain constructs and genes studied

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype or construct</th>
<th>Morphotype or antibiotic resistance</th>
<th>Relevant features</th>
<th>Reference</th>
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<td><strong>E. coli TOB1 derivatives</strong></td>
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<td>TOB1 Wild-type</td>
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<td>rdar&lt;sub&gt;28&lt;/sub&gt;C/rdar&lt;sub&gt;37&lt;/sub&gt;C</td>
<td>Cellulose&lt;sup&gt;+&lt;/sup&gt;; curli fimbriae&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This study</td>
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<td>TOB2 TOB1 csgD::Cm</td>
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<td>saw&lt;sub&gt;28&lt;/sub&gt;C/saw&lt;sub&gt;37&lt;/sub&gt;C</td>
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<td>TOB2p TOB1 csgD::Cm; CsgD&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
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<td>Cellulose&lt;sup&gt;+&lt;/sup&gt;; curli fimbriae&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>ATCC 14028</td>
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<td>MAE52 UMR1 PcsgD&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>Cellulose&lt;sup&gt;+&lt;/sup&gt;; curli fimbriae&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Römling et al. (1998a)</td>
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<tr>
<td>MAE51 MAE52 ΔcsgD</td>
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<td>saw&lt;sub&gt;28&lt;/sub&gt;C/saw&lt;sub&gt;37&lt;/sub&gt;C</td>
<td>Cellulose&lt;sup&gt;+&lt;/sup&gt;; curli fimbriae&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>pUMR15 pBAD30::csgD</td>
<td></td>
<td>Amp</td>
<td>Arabinose-dependent expression of csgD</td>
<td>Römling et al. (2000)</td>
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</table>

RESULTS

Colony morphology and phylogenetic characterization of faecal E. coli isolates

Fifty-two E. coli biotypes were found from the 115 E. coli faecal isolates collected from 21 individuals according to the criteria described in Methods. Serotyping revealed a high number of O-non-typable (Ont) strains as is characteristic for commensal isolates (Orskov, 1978) (Table 2 and data not shown). Colony morphotypes were assessed on agar plates, and representative morphotypes are shown in Fig. 1a. Eight different morphotypes were found (mean of 2.2 morphotypes per individual), with 74 % of the isolates belonging to the four dominant morphotypes: rdar<sub>28</sub>C/rdar<sub>37</sub>C (11 isolates); brown, dry and rough (bdar) morphotype. Finally, in the rdar morphotype, both components are expressed (Römling et al., 2000). Since the rdar morphotype has been associated with virulence (Bian et al., 2000), we used samples from UTI infections as a control. Ninety-eight isolates resulting in 24 representative strains (1-6 morphotypes per individual) were chosen in the same way as described for the faecal strains. Serotyping revealed that almost all strains showed O-serotypes characteristic for isolates from UTIs or were rough (Orskov, 1978) (data not shown). Eleven different morphotypes (Fig. 1b) could be discriminated between the isolates, with a different morphotype distribution in comparison to the faecal isolates. A subgroup of those morphotypes has recently been characterized on the molecular level (Zogaj et al., 2003).

Colony morphology of other Enterobacteriaceae isolated from the gastrointestinal tract

In several individuals, other enterobacterial species besides E. coli were discovered (Table 3). In an individual sample, the proportion of colonies not belonging to E. coli was in the range from 3 to 66 %. A variety of species-specific morphotypes were discovered among the isolates (Table 3). A subgroup of those morphotypes has recently been characterized on the molecular level (Zogaj et al., 2003).

Colony morphology of isolates from UTIs

Since the rdar morphotype has been associated with virulence (Bian et al., 2000), we used samples from UTI infections as a control. Ninety-eight isolates resulting in 24 representative strains (1-6 morphotypes per individual) were chosen in the same way as described for the faecal strains. Serotyping revealed that almost all strains showed O-serotypes characteristic for isolates from UTIs or were rough (Orskov, 1978) (data not shown). Eleven different morphotypes (Fig. 1b) could be discriminated between the isolates, with a different morphotype distribution in comparison to the faecal isolates. The most frequent morphotypes were rdar/saw, which was recovered six times, and saw/saw, which was recovered four times.

Expression of curli fimbriae and cellulose by various morphotypes

In S. Typhimurium, expression of curli creates the bdar morphotype. In contrast, expression of cellulose creates the pink, dry and rough (pdar) morphotype. Finally, in the rdar morphotype, both components are expressed (Römling et
<table>
<thead>
<tr>
<th>Subject (family, type*, age)</th>
<th>E. coli isolates (percentage of total isolates)†</th>
<th>Phenotype on CR plates (at 28 °C/37 °C)</th>
<th>Frequency (percentage of E. coli morphotype)</th>
<th>Strain</th>
<th>Phyllogenetic group</th>
<th>PFGE type</th>
<th>Serotype</th>
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<td>Fec81</td>
<td>D</td>
<td>O</td>
<td>O44 : H18</td>
<td>P fimbrion; class II; aerolysin</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Fec93</td>
<td>A</td>
<td>O1</td>
<td>O128 : H2</td>
<td>—</td>
</tr>
<tr>
<td>F2, F, 33</td>
<td>100</td>
<td></td>
<td></td>
<td>Fec91</td>
<td>D</td>
<td>O</td>
<td>O44 : H18</td>
<td>P fimbrion; class II; aerolysin</td>
</tr>
<tr>
<td>F2, C, 5</td>
<td>100</td>
<td></td>
<td></td>
<td>Fec95</td>
<td>B1</td>
<td>O4</td>
<td>O128 : H2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fec97</td>
<td>A</td>
<td>O5</td>
<td>O63 : H—</td>
<td>—</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Fec98</td>
<td>A</td>
<td>Q</td>
<td>O44 : H18</td>
<td>—</td>
</tr>
<tr>
<td>F2, C, 1-9</td>
<td>97</td>
<td></td>
<td></td>
<td>Fec113</td>
<td>B1</td>
<td>R2</td>
<td>O174 : H16</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fec101</td>
<td>B1</td>
<td>R</td>
<td>Ont : H16</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fec102</td>
<td>B1</td>
<td>R</td>
<td>O174 : H2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fec112</td>
<td>B1</td>
<td>R1</td>
<td>Ont : H16</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fec108</td>
<td>B1</td>
<td>S</td>
<td>Ont : H25</td>
<td>P fimbrion; aerolysin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fec109</td>
<td>B1</td>
<td>S</td>
<td>O9 : H25</td>
<td>P fimbrion; aerolysin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fec110</td>
<td>D</td>
<td>T</td>
<td>Ont : Hrough</td>
<td>—</td>
</tr>
</tbody>
</table>
To test this correlation, 34 faecal *E. coli* isolates from the three families and 18 isolates from UTIs with representative morphotypes were examined.

Expression of curli fimbriae was detected as described in Methods (Fig. 3). Expression of curli fimbriae was commonly correlated with the expression of the rdar (ras) and bdar (bas) morphotypes (Fig. 1, Table 4), whereby the level of expression of curli fibres could vary as described elsewhere (Bian *et al.*, 2000). However, high expression of cellulose could disguise low expression of curli fimbriae, resulting in an apparent pdar morphotype.

Expression of cellulose was tested by screening colonies for Calcofluor binding. While expression of curli fibres gave a low background fluorescence, specific Calcofluor binding was only detected when isolates showed rdar (ras) or pdar (pas) colony morphologies (Fig. 1, Table 4). Cellulose was isolated from selected isolates, and the glucose monomers were detected after hydrolysis as previously described (Römling *et al.*, 2003). Detection of cellulose production correlated with Calcofluor binding and the rdar/pdar morphotype (Table 4).

At 28 °C, 14 of the 52 faecal isolates expressed only curli fimbriae, 27 expressed both matrix components and 11 did not express either matrix component. At 37 °C, one isolate expressed cellulose, 12 expressed only curli and 11 expressed...
both matrix components. One of the 24 UTI isolates expressed only cellulose at 28 °C, whilst four expressed only curli and 11 expressed both matrix components. At 37 °C, five UTI isolates expressed only cellulose, five only curli and one both components. In general, commensal isolates typically expressed cellulose and curli fimbriae at 28 °C and 37 °C, while UTI isolates typically expressed cellulose and curli fimbriae at 28 °C.

**Construction of mutants with distinct expression of extracellular matrix components**

The representative faecal isolate TOB1, which expresses curli fimbriae and cellulose at 28 °C and 37 °C, was chosen for further genetic analysis of regulation of extracellular matrix components. TOB1 belongs to the phylogenetic group B2, which harbours pathogenic as well as non-pathogenic strains (Duriez et al., 2001). However, PCR tests showed that TOB1 did not harbour common virulence factors, did not express haemolytic activity and was a prototroph (Table 2, data not shown). In addition, TOB1 did not show signs of virulence when colonizing germ-free mice for 2 weeks (data not shown). Isogenic mutants for the individual matrix components were constructed using TOB1 as a parent strain and the phenotype was assayed on agar plates (Fig. 4, Table 1). When the transcriptional regulator csgD was knocked out, expression of both extracellular matrix components was abolished in strain TOB2. A colony with a saw morphotype was observed, indicating that no matrix components were produced (Fig. 4). Complementation of the ΔcsgD mutant with csgD on a plasmid (pUMR15) resulted in high cellulose biosynthesis only (pdar morphotype in strain TOB2p), since the knockout of csgD had a polar effect on the genes downstream in the csgDEFG operon, which are required for curli biosynthesis. Knockout of bcsA, encoding the catalytic subunit of cellulose synthase, abolished cellulose biosynthesis in strain TOB3 and resulted in the production of only curli (bdar morphotype; Fig. 4). Therefore, the regulatory and structural genes for the production of the extracellular matrix are equivalent in S. Typhimurium and E. coli.

**Table 3. Percentages and phenotypes of Enterobacteriaceae isolated from human faeces**

<table>
<thead>
<tr>
<th>Subject (family, type*, age)</th>
<th>Non-E. coli isolates (percentage of total isolates)</th>
<th>Species</th>
<th>Frequency (percentage of total isolates)</th>
<th>Phenotype on Congo red plates (at 28 °C/37 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1, M, 28</td>
<td>65</td>
<td>Enterobacter cloacae</td>
<td>52</td>
<td>saw/pas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterobacter sakazakii</td>
<td>13</td>
<td>pdar/pdar</td>
</tr>
<tr>
<td>F1, C, 7</td>
<td>62</td>
<td>Klebsiella oxytoca</td>
<td>62</td>
<td>mucoid/mucoid</td>
</tr>
<tr>
<td>F1, C, 4</td>
<td>66</td>
<td>Klebsiella pneumoniae</td>
<td>66</td>
<td>mucoid/mucoid</td>
</tr>
<tr>
<td>F2, C, 1-9</td>
<td>3</td>
<td>Citrobacter freundii</td>
<td>2</td>
<td>rdar/saw</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrobacter spp.</td>
<td>1</td>
<td>bdar/saw</td>
</tr>
<tr>
<td>F3, M, 45</td>
<td>66</td>
<td>Enterobacter cloacae</td>
<td>60</td>
<td>saw/saw</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterobacter cloacae</td>
<td>4</td>
<td>saw/pas</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kluyvera spp.</td>
<td>2</td>
<td>saw/saw</td>
</tr>
<tr>
<td>F3, C, 0-4</td>
<td>3·2</td>
<td>Raoultella ornithinolytica</td>
<td>2</td>
<td>saw/saw</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrobacter spp.</td>
<td>1</td>
<td>bdar/saw</td>
</tr>
</tbody>
</table>

*C, child; F, female; M, male.

**Biofilm formation of faecal and UTI isolates with different morphotypes**

Next, we assayed production of biofilms by TOB1 and its mutants (Fig. 5). At 28 °C and 37 °C, co-expression of cellulose and curli fimbriae in TOB1 resulted in significant biofilm formation (pellicle formation, clumping and adherence to the wall of wells) as indicated by a clear broth. Deletion of matrix components reduced biofilm formation. At 28 °C, the deletion of curli abolished adherence to the wall of wells and biofilm formation (TOB2 and TOB2p). Clumping was still observed when only cellulose was expressed (TOB2p). Surprisingly, biofilm formation was independent of both extracellular matrix components at 37 °C (data not shown). This latter finding contrasts the observation made in S. Typhimurium ATCC 14028 (Römling et al., 2000). TOB1 also formed intermediate level of biofilm in minimal medium (Fig. 6).

Nineteen isolates with distinct morphotypes on CR agar plates were chosen to investigate the capacity of faecal strains to form biofilms in nutrient rich and poor media, LB without...
Table 4. Morphotype and expression of extracellular matrix components

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Morphotype on Congo red plates (at 28 °C/37 °C)</th>
<th>Calcofluor binding† (at 28 °C/37 °C)</th>
<th>CsgA expression‡ (at 28 °C/37 °C)</th>
<th>Cellulose expression (at 28 °C/37 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOB1</td>
<td>rdar/rdar</td>
<td>++/ +</td>
<td>+/ +</td>
<td>ND</td>
</tr>
<tr>
<td>Fec5</td>
<td>rdar/pdar</td>
<td>+/ +</td>
<td>+/(+)</td>
<td>+/ +</td>
</tr>
<tr>
<td>Fec6</td>
<td>bdar/saw</td>
<td>B/--</td>
<td>++/--</td>
<td>ND</td>
</tr>
<tr>
<td>Fec10</td>
<td>rdar/saw</td>
<td>+/--</td>
<td>++/--</td>
<td>+/--</td>
</tr>
<tr>
<td>Fec12</td>
<td>rdar/rdar</td>
<td>++/ +</td>
<td>+/+/+++</td>
<td>ND</td>
</tr>
<tr>
<td>Fec23</td>
<td>rdar/pdar</td>
<td>++/ +</td>
<td>+/+++</td>
<td>ND</td>
</tr>
<tr>
<td>Fec26</td>
<td>rdar/pas</td>
<td>+/+</td>
<td>+/+++</td>
<td>ND</td>
</tr>
<tr>
<td>Fec27</td>
<td>bdar/saw</td>
<td>--/ --</td>
<td>--/ --</td>
<td>ND</td>
</tr>
<tr>
<td>Fec28</td>
<td>bdar/saw</td>
<td>B/--</td>
<td>+/--</td>
<td>ND</td>
</tr>
<tr>
<td>Fec41</td>
<td>rdar/bas</td>
<td>+/-</td>
<td>+/+++</td>
<td>+/§</td>
</tr>
<tr>
<td>Fec51</td>
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<td>B/B</td>
<td>++/+</td>
<td>+/ND</td>
</tr>
<tr>
<td>Fec53</td>
<td>saw/saw</td>
<td>--/ --</td>
<td>--/ --</td>
<td>ND</td>
</tr>
<tr>
<td>Fec55</td>
<td>bdar/bas</td>
<td>B/--</td>
<td>+/--</td>
<td>ND/ --</td>
</tr>
<tr>
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<td>+/-</td>
<td>+/+++</td>
<td>+/+</td>
</tr>
<tr>
<td>Fec61</td>
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<td>--/ --</td>
<td>--/ --</td>
<td>ND</td>
</tr>
<tr>
<td>Fec65</td>
<td>bdar/bdar</td>
<td>--/ --</td>
<td>++/++</td>
<td>ND/ --</td>
</tr>
<tr>
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<td>+++/++</td>
<td>+/+</td>
</tr>
<tr>
<td>Fec69</td>
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<td>B/--</td>
<td>++/(+)</td>
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<tr>
<td>Fec73</td>
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<td>--/ --</td>
<td>--/ --</td>
<td>ND</td>
</tr>
<tr>
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<td>B/--</td>
<td>++/+</td>
<td>--/ --</td>
</tr>
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</tr>
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<td>+/+++</td>
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</tr>
<tr>
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<td>bdar/saw</td>
<td>B/--</td>
<td>++/(+)</td>
<td>--/ND</td>
</tr>
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<td>+/--</td>
<td>++/--</td>
<td>ND</td>
</tr>
<tr>
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<td>B/B</td>
<td>++/+</td>
<td>ND</td>
</tr>
<tr>
<td>Fec98</td>
<td>pdar/saw</td>
<td>+/B</td>
<td>++/--</td>
<td>+/ --</td>
</tr>
<tr>
<td>Fec99</td>
<td>pdar/saw</td>
<td>+/-</td>
<td>++/--</td>
<td>ND</td>
</tr>
<tr>
<td>Fec100</td>
<td>rdar/saw</td>
<td>+/-</td>
<td>++/--</td>
<td>ND</td>
</tr>
<tr>
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<td>+/-</td>
<td>++/++</td>
<td>+/+</td>
</tr>
<tr>
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<td>rdar/rdar</td>
<td>+/-</td>
<td>++/++</td>
<td>ND/ +ND</td>
</tr>
<tr>
<td>Fec109</td>
<td>bdar/saw</td>
<td>--/ --</td>
<td>++/--</td>
<td>ND</td>
</tr>
<tr>
<td>Fec110</td>
<td>pdar/pdar</td>
<td>+/-</td>
<td>++/++</td>
<td>ND</td>
</tr>
<tr>
<td>Fec112</td>
<td>rdar/rdar</td>
<td>+/-</td>
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<td>B/B</td>
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<td>ND</td>
</tr>
<tr>
<td>Uti1</td>
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<td>--/ --</td>
<td>+§/ --</td>
</tr>
<tr>
<td>Uti5</td>
<td>saw/saw</td>
<td>--/ --</td>
<td>--/ --</td>
<td>--/ --</td>
</tr>
<tr>
<td>Uti13</td>
<td>rdar/bas</td>
<td>B/B</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Uti19</td>
<td>bdar/saw</td>
<td>B/--</td>
<td>B/--</td>
<td>ND</td>
</tr>
<tr>
<td>Uti20</td>
<td>rdar/saw</td>
<td>+/--</td>
<td>++/--</td>
<td>ND</td>
</tr>
<tr>
<td>Uti22</td>
<td>rdar/saw</td>
<td>+/--</td>
<td>++/--</td>
<td>ND</td>
</tr>
<tr>
<td>Uti26</td>
<td>pdar/pas</td>
<td>+/ +</td>
<td>++/++/--</td>
<td>ND</td>
</tr>
<tr>
<td>Uti28</td>
<td>pdar/pdar</td>
<td>+/-</td>
<td>++/+</td>
<td>--/§</td>
</tr>
<tr>
<td>Uti30</td>
<td>pdar/pas</td>
<td>+/-</td>
<td>++/±</td>
<td>+/– §</td>
</tr>
<tr>
<td>Uti40</td>
<td>saw/saw</td>
<td>--/ --</td>
<td>--/ --</td>
<td>ND</td>
</tr>
<tr>
<td>Uti41</td>
<td>saw/saw</td>
<td>--/ --</td>
<td>+/–</td>
<td>ND</td>
</tr>
<tr>
<td>Uti43</td>
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<td>--/ --</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Uti45</td>
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<td>++/++</td>
<td>ND</td>
</tr>
<tr>
<td>Uti49</td>
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<td>+/-</td>
<td>+/–</td>
<td>ND</td>
</tr>
<tr>
<td>Uti55</td>
<td>rdar/saw</td>
<td>+/-</td>
<td>+/±</td>
<td>++/(+)</td>
</tr>
</tbody>
</table>
salt and M9 minimal medium. UTI isolates and control strains with known biofilm behaviour were also used. Most of the faecal and UTI strains showed only a low capacity to form biofilms in nutrient-defined medium (Fig. 6). However, biofilm formation in LB medium without salt correlated with the colony morphotype on CR agar plates. Strains expressing the rdar (ras) morphotype showed medium or high biofilm-formation capacity, while strains expressing the saw morphotype only displayed medium biofilm-forming capacity. Bdar (bas) and pdar (pas) morphotype strains showed a lower capacity to form biofilms than rdar (ras) strains. In summary, with the exception of strains displaying the saw morphotype, all strains expressing matrix components showed medium or high biofilm-forming capacity under at least one of the growth conditions tested.

**DISCUSSION**

In this communication, we report the ability of primary commensal isolates of *E. coli* to express curli fimbriae and cellulose on LB agar without salt, which is coupled to the biofilm-formation capability of the organism. Only *Salmo nella* serotypes had been systematically examined for the occurrence of the rdar morphotype, with the vast majority of *S. Typhimurium* and *Salmonella enterica* serovar Enteritidis strains showing low level expression of both extracellular matrix components at ambient temperatures (rdar/saw morphotype) (Römling *et al*., 2003). The rdar/saw morphotype was expressed by five strains among commensal isolates of *E. coli*, but those strains were present at very low frequency (0·33%), while rdar/saw was the most frequent morphotype.

### Table 4. cont.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Morphotype on Congo red plates (at 28 °C/37 °C)</th>
<th>Calcofluor binding† (at 28 °C/37 °C)</th>
<th>CsgA expression‡ (at 28 °C/37 °C)</th>
<th>Cellulose expression (at 28 °C/37 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uti57</td>
<td>rdar/saw</td>
<td>+/B</td>
<td>++/−</td>
<td>+/−</td>
</tr>
<tr>
<td>Uti59</td>
<td>rdar/saw</td>
<td>B/−</td>
<td>(+)/−</td>
<td>ND</td>
</tr>
<tr>
<td>Uti83</td>
<td>saw/bas</td>
<td>−/B</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Uti85</td>
<td>pas/pas</td>
<td>+/+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Uti92</td>
<td>pas/bdar</td>
<td>+/B</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not done.

*Strains underlined were investigated in the biofilm formation assay shown in Fig. 6.

†Binding of Calcofluor: +, significant fluorescence (comparable to *S. Typhimurium* MAE52 and MAE97); −, no fluorescence (comparable to *S. Typhimurium* MAE51); B, background level of fluorescence (comparable to *S. Typhimurium* AdrA 1a). Reference phenotypes see Fig. 1a.

‡CsgA expression varied from no expression ‘−’ to high expression ‘+++’ using *S. Typhimurium* MAE51 (−) and *S. Typhimurium* MAE52 (+++ as references in protein gel, Western blot and MALDI-TOF analysis.’(+)’ Represents a faint signal, positive result not achieved by all three methods used.

§Discrepancy between phenotype and cellulose expression assay.

---

**Fig. 4.** Morphotypes and regulation of extracellular matrix mutants of *E. coli* TOB1. Morphotypes are: TOB1, rdar; TOB2, saw; TOB3, bdar; TOB2p, pdar. Cells were grown on CR agar plates for 48 h at 28 °C and for 24 h at 37 °C.
among UTI strains (25%). Highly regulated rdar morphotype expression seems to be a characteristic of a subset of enterobacterial pathovars (Olsen et al., 1993; Römling et al., 2003; Sjöbring et al., 1994; Zogaj et al., 2003). To summarize, in contrast to Salmonella serovars, variation of expression of curli fimbriae and cellulose was common among commensal E. coli strains, with eight morphotypes discriminated.

In the representative isolate E. coli TOB1 and other faecal and UTI isolates expression of matrix components contributed to biofilm formation as demonstrated previously (Austin et al., 1998; Römling et al., 1998b; Zogaj et al., 2001). Thus, rdar morphotype expression on CR agar plates is an indicator of biofilm formation. However, a pellicle was formed independently of cellulose and curli fimbriae by TOB1 at 37 °C. E. coli expresses other candidate polysaccharides and fimbriae contributing to biofilm formation (Pratt & Kolter, 1998; Wang et al., 2004). Yet, the human gastrointestinal tract is a major source of biofilm-forming E. coli isolates, which might spread to susceptible sites where they can cause infection (for example, catheter-related UTIs).

At 37 °C a significant percentage of isolates (44%) expressed both extracellular matrix components (21%) or only curli fimbriae (23%). As such, these are characteristics of commensal isolates. Therefore, it is reasonable to expect an in vivo role for curli fimbriae in commensal–host interaction. Invasion of host cells has been shown to be mediated by curli fimbriae (Gophna et al., 2001; Uhlich et al., 2002).

Expression of curli fimbriae and/or cellulose does not seem to be a prerequisite for colonization of the human gastrointestinal tract. However, the definitive role of curli fimbriae and/or cellulose in colonization can only be elucidated after the
use of knockout mutants, since also isolates phenotypically negative by plate-growth might be able to express the matrix components in the host. In any case, a variety of different expression patterns of extracellular matrix components are found among the commensal isolates, which indicates different regulatory mechanisms controlling the expression of cellulose and curli fimbriae.

Within an individual, there was usually more than one morphotype (Table 2). Frequently, different morphotypes within an individual and among family members belonged to the same pulsed-field type. These data indicate that clones diversify morphotypically within the human gastrointestinal tract. It remains to be shown whether strains with different morphotypes occupy different niches within the gastrointestinal tract.

Type 1 fimbriae and curli fimbriae are the only two fimbriae commonly present in commensal and pathogenic E. coli strains, whereby regulation by environmental conditions is different (Blomfield, 2001; Gerstel & Romling, 2003). For type 1 fimbriae, allelic variation of the tip adhesin FimH leads to tissue tropism of commensals and pathogens (Sukukeno et al., 1998). Whether curli fimbriae expressed by commensals and pathogens also show such discriminatory binding remains to be shown.

Phenotypes were more diverse among UTI isolates as compared to commensal isolates (11 and eight morphotypes, respectively). Morphotypes that displayed only cellulose, but not curli fimbriae, as an extracellular matrix component occurred almost exclusively in UTI isolates (Fig. 1b), suggesting that selective loss of curli expression might occur in the urinary tract. Although not statistically significant, the rdar/saw morphotype was typical for UTI isolates, while rdar/rdar was typical for commensal isolates.

In any case, almost nothing is known about the features of commensal E. coli strains. Investigation of this strain population will contribute to our understanding of the important role the commensal flora plays in human health.

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REFERENCES


Lancet 361, 512–519.

Expression of two csg operons is required for production of fibronectin- 
and congo red-binding curli polymers in Escherichia coli K-12. Mol 
Microbiol 18, 661–670.


Herwald, H., Morgelin, M., Olsen, A., Rhen, M., Dahlback, B., Muller- 
Esterl, W. & Bjorck, L. (1998). Activation of the contact-phase system on 
bacterial surfaces – a clue to serious complications in infectious 

Colonization with and acquisition of uropathogenic Escherichia coli as 
revealed by polymerase chain reaction-based detection. J Infect Dis 177, 
1120–1124.

Advances in the bacteriology of the coliform group: their suitability as 

Type 1 pili are not necessary for colonization of the streptomycin- 

and aerobactin characterize colonic resident Escherichia coli. Epidemiol 
Infect 126, 11–18.

O’Boyle, C. J., MacFie, J., Mitchell, C. J., Johnstone, D., Sagar, P. M. & 
Sedman, P. C. (1998). Microbiology of bacterial translocation in 

meditated by a novel class of surface organelles on Escherichia coli. Nature 

Olsen, A., Arnvist, A., Hammar, M., Sukupolvi, S. & Normark, S. 
(1993). The RpoS sigma factor relieves H-NS-mediated transcriptional 
regulation of csgA, the subunit gene of fibronectin-binding curli in 

Olsen, A., Herwald, H., Wikstrom, M., Persson, K., Mattsson, E. & 
regions of curli, a surface organelle and virulence determinant of 

Dis 137, 630–633.

on urinary Escherichia coli isolates. In Genes and Proteins Underlying 
Microbial Urinary Tract Virulence, pp. 219–224. Edited by L. Emody. 
Dordrecht: Kluwer/Plenum.

formation: roles of flagella, motility, chemotaxis and type I pili. Mol 


Römling, U., Fiedler, B., Bosshammer, J., Grothues, D., Greipel, J., von 
Pseudomonas aeruginosa infections in cystic fibrosis. J Infect Dis 170, 
1616–1621.

Römling, U., Bian, Z., Hammar, M., Sierralta, W. D. & Normark, S. 
(1998a). Curli fibers are highly conserved between Salmonella typhi- 
murium and Escherichia coli with respect to operon structure and 

Multicellular and aggregative behaviour of Salmonella typhimurium 
strains is controlled by mutations in the agfD promoter. Mol Microbiol 
28, 249–264.

AgfD, the checkpoint of multicellular and aggregative behaviour in 
Salmonella typhimurium regulates at least two independent pathways. Mol 
Microbiol 36, 10–23.

Römling, U., Bokranz, W., Rabsch, W., Zogaj, X., Nimtz, M., Tschäpe, H. 
(2003). Occurrence and regulation of the multicellular morphotype in 

Sakellaris, H., Hannink, N. K., Rajakumar, K., Bulach, D., Hunt, M., 

Escherichia coli expressing curli or by Salmonella enteritidis expressing 
thin aggregative fimbriae, can be activated by simultaneously captured 
tissue-type plasminogen activator (t-PA). Mol Microbiol 14, 443–452.

Struelens, M. J. (1996). Consensus guidelines for appropriate use and 
evaluation of microbial epidemiologic typing systems. Clin Microbiol 
Infect 2, 2–11.

Sukurenko, E. V., Chesnokova, V., Dykhuizen, D. E., Ofek, I., Wu, Z.-R., 
Pathogenic adaptation of Escherichia coli by natural variation of the 

promoter associated with variations in curli expression in certain strains 

promoter of Escherichia coli O157:H7 associated with increased 
virulence in mice and increased invasion of HEp-2 cells. Infect Immun 
70, 395–399.


Escherichia coli promotes the synthesis of a polysaccharide adhesin 

Wold, A. E., Caugant, D. A., Lidin-Janson, G., de Man, P. & Svanborg, C. 
(1992). Resident colonic Escherichia coli strains frequently display 

The multicellular morphotypes of Salmonella typhimurium and Escherichia 
coli produce cellulose as the second component of the extracellular 

curli and fibronectin by members of the family Enterobacteriaceae 
isolated from the human gastrointestinal tract. Infect Immun 71, 4151– 
4158.