Asymptomatic bacteriuria *Escherichia coli* strain 83972 carries mutations in the *foc* locus and is unable to express F1C fimbriae

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Escherichia coli is the most common organism associated with asymptomatic bacteriuria (ABU). In contrast to uropathogenic *E. coli* (UPEC), which causes symptomatic urinary tract infection (UTI), very little is known about the mechanisms by which these strains colonize the urinary tract. Bacterial adhesion conferred by specific surface-associated adhesins is normally considered as a prerequisite for colonization of the urinary tract. The prototype ABU *E. coli* strain 83972 was originally isolated from a girl who had carried it asymptotically for 3 years. This study characterized the molecular status of one of the primary adhesion factors known to be associated with UTI, namely F1C fimbriae, encoded by the *foc* gene cluster. F1C fimbriae recognize receptors present in the human kidney and bladder. Expression of the *foc* genes was found to be up-regulated in human urine. It was also shown that although strain 83972 contains a seemingly intact *foc* gene cluster, F1C fimbriae are not expressed. Sequencing and genetic complementation revealed that the *focD* gene, encoding a component of the F1C transport and assembly system, was non-functional, explaining the inability of strain 83972 to express this adhesin. The data imply that *E. coli* 83972 has lost its ability to express this important colonization factor as a result of host-driven evolution. The ancestor of the strain seems to have been a pyelonephritis strain of phylogenetic group B2. Strain 83972 therefore represents an example of bacterial adaptation from pathogenicity to commensalism through virulence factor loss.

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INTRODUCTION

Urinary tract infections (UTIs) are among the most common infectious diseases of humans and a major cause of morbidity and mortality. It is estimated that 40–50 % of healthy adult women have experienced at least one UTI episode (Foxman, 2002). Acute pyelonephritis and asymptomatic bacteriuria (ABU) represent the two extremes of UTI. Acute pyelonephritis is a severe acute systemic infection caused by uropathogenic *Escherichia coli* (UPEC) clones with virulence genes clustered on ‘pathogenicity islands’ (Eden *et al.*, 1976; Funfstuck *et al.*, 1986; Johnson, 1991; Orskov *et al.*, 1988; Stenqvist *et al.*, 1987; Welch *et al.*, 2002). ABU, on the other hand, is an asymptomatic carrier state that resembles commensalism. ABU patients may carry $>10^5$ c.f.u. ml⁻¹ of a single *E. coli* strain for months or years without provoking a host response. In early studies

this was explained by a lack of virulence genes, as the majority of ABU-associated *E. coli* strains are non-haemolytic, non-adherent and lack haemagglutination ability (Eden *et al.*, 1976; Kaijser & Ahlstedt, 1977; Lindberg *et al.*, 1975). Molecular epidemiology has shown, however, that >60 % of ABU strains carry virulence genes even though they fail to express the phenotype (Plos *et al.*, 1990, 1995).

The ability of UPEC to cause symptomatic UTI is enhanced by adhesins, e.g. F1C, P and type 1 fimbriae (Klemm & Schembri, 2000; Oelschlaeger *et al.*, 2002). Tissue surfaces in the urinary tract are submitted to strong hydrodynamic shear forces. Adherence to the urinary tract epithelium enables the bacteria to resist the hydrodynamic forces of urine flow and to establish infection. F1C fimbriae are expressed by a large proportion of urinary tract infectious *E. coli* strains; depending on the study 14–30 % of UTI strains have been reported to be able to express these fimbriae (Pere *et al.*, 1987; Siitonen *et al.*, 1993). F1C fimbriae, like P and type 1 fimbriae, are surface polymers 7 nm wide and approximately 1 µm long. The bulk of an F1C fimbria is made up of about 1000 subunits of a major

Abbreviations: ABU, asymptomatic bacteriuria; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

The GenBank/EMBL/DDBJ accession number for the *E. coli* strain 83972 *foc* gene cluster is DQ301498.

building element, the FocA protein. Additionally, a few copies of minor components, FocF, FocG and FocH, are integral parts of the fimbria, and are responsible for its adhesive properties. Export of the structural components of the fimbriae to the surface depends on a periplasmic chaperone, FocC, and an outer-membrane-located usher, FocD (Klemm *et al.*, 1994, 1995). F1C fimbriae specifically recognize galatosylceramide targets present on epithelial cells in the kidneys, ureters and bladder as well as globotriaosylceramide, present only in the kidneys (Bäckhed *et al.*, 2002; Khan *et al.*, 2000). Recently, it was shown that human renal epithelial cells specifically produce the proinflammatory cytokine IL-8 in response to F1C-mediated attachment (Bäckhed *et al.*, 2002).

The ABU strain *E. coli* 83972 is a clinical isolate capable of long-term bladder colonization. The strain was originally isolated from a young Swedish girl with ABU who had carried it for at least 3 years without symptoms (Andersson *et al.*, 1991; Lindberg *et al.*, 1975). It is well adapted for growth in the urinary tract, where it establishes long-term bacteriuria (Andersson *et al.*, 1991; Hull *et al.*, 2000; Wullt *et al.*, 1998, 2000). The strain has successfully been used for prophylactic purposes in patients with recurrent UTI. Here the bladders of patients are deliberately colonized with strain 83972 in order to prevent the establishment of UPEC stains. The ability of *E. coli* 83972 to establish efficient long-term colonization of the human bladder without evoking countermeasures from the host defences is not fully understood. The strain has been reported to carry genes of the *pap*, *fim* and *foc* gene clusters, encoding P, type 1 and F1C fimbriae, respectively (Hull *et al.*, 1999). We have recently demonstrated that the strain is unable to express functional P and type 1 fimbriae (Klemm *et al.*, 2006). In this study we investigate the status of the *foc* genes and corresponding F1C fimbriae in this interesting strain.

METHODS

Bacterial strains, plasmids and growth conditions. The strains and plasmids used in this study are described in Table 1. *E. coli* 83972 is a prototype ABU strain and lacks defined O and K surface antigens. It carries adhesin gene clusters homologous to *fim*, *pap* and *foc* but does not seem to express functional fimbrial adhesins after *in vitro* culture or when recovered from the urinary tract (Andersson *et al.*, 1991; Hull *et al.*, 1999). The 83972 *pap::kan* mutant was constructed using the λ Red recombinase gene replacement system (Datsenko & Wanner, 2000). Briefly, the *npt* gene from plasmid pKD4 was amplified by primers containing 50-nucleotide *pap* homology extensions (*papf*, 5'-CAGGGCCAGGGAGAAGTAACTTTCAAAGGAAGTGTGTTGACGCTCCATGGTGTAGGCTGGAGCTGCTTC-3' and *papr*, 5'-CCTTTGTCCACGCCATTAACCGAAACAATGGAGTCCCAGCC-ATGACCCAGCATATGAATATCCTCCTTA-3'). This product was digested with *DpnI* and transformed into 83972(pKD46), and kanamycin-resistant colonies were selected. The λ Red helper plasmid pKD46 was cured by growth at 37 °C and the correct double-crossover and recombination event was confirmed by PCR and DNA sequencing. This strain, designated 83972*pap*, therefore contains a deletion in the region spanning the *papA-papG* genes. Cells were routinely grown at 37 °C on solid or in liquid Luria-Bertani (LB) medium supplemented with the appropriate antibiotics unless otherwise stated.

DNA manipulations and genetic techniques. Plasmid DNA was isolated using the QIAprep Spin Miniprep kit (Qiagen). Restriction endonucleases were used according to the manufacturer's specifications (Biolabs). Chromosomal DNA was purified using the GenomicPrep Cell and Tissue DNA isolation kit (Amersham Biosciences). All PCRs were performed with the Expand High Fidelity Polymerase System or the Expand Long Template PCR system according to the manufacturer's instructions (Roche). pPKL332 was constructed from pPKL160 by digestion with *KpnI*, followed by T4 DNA polymerase treatment and religation. DNA sequencing of the PCR amplified *foc* gene cluster was performed using primer walking technology. Sequencing reactions were carried out by MWG Biotech.

Agglutination assays. The capacity of bacteria to express hybrid F1C fimbriae with incorporated FimH was assayed by their ability

Table 1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Reference
<i>E. coli</i> strains		
83972	ABU isolate (OR:K5:H ⁻)	Andersson <i>et al.</i> (1991)
83972 <i>pap</i>	ABU 83972 Δ <i>pap</i>	This study
MG1655	K-12 reference strain	Bachmann (1996)
MS428	MG1655 Δ <i>fim</i>	Kjaergaard <i>et al.</i> (2000)
Plasmids*		
pPIL110-541 (B)	<i>focA</i>	van Die <i>et al.</i> (1985)
pPKL136 (U)	<i>focC</i>	Klemm <i>et al.</i> (1995)
pPKL143 (B)	All <i>foc</i> genes	Klemm <i>et al.</i> (1994)
pPKL148 (A)	<i>focFGH</i>	Klemm <i>et al.</i> (1994)
pPKL160 (A)	<i>focCD</i>	Klemm <i>et al.</i> (1995)
pPKL163 (B)	All <i>foc</i> genes except <i>focD</i>	Klemm <i>et al.</i> (1995)
pPKL332 (A)	<i>focD</i>	This study

*A, B and U indicate pACYC184, pBR322 and pUC, respectively.

to agglutinate yeast (*Saccharomyces cerevisiae*) cells on glass slides. Aliquots of washed bacterial suspensions at OD₆₀₀ 0.5 and 5% yeast cells were mixed and the time until agglutination occurred was measured.

Western immunoblotting. For maximized expression of F1C fimbriae, cells were grown in 100 ml filter-sterilized pooled human urine. Fimbrial proteins detached from the surface of the cells by blending and proteins from total cell lysates were separated on 15% polyacrylamide gels by SDS-PAGE. The proteins were then transferred to PVDF membranes. The membranes were blocked with 0.5% Tween-20 and incubated with anti-F1C fimbriae serum, which was a generous gift from Timo Korhonen (University of Helsinki, Finland) and Ulrich Dobrindt (University of Würzburg, Germany). The membrane was then incubated with horseradish-peroxidase-labelled antibodies followed by visualization with tetramethylbenzidine (TMB).

Microarray analysis. The microarray results of the *foc* cluster are extracted from a previously published complete genome microarray study of *E. coli* 83972; the experimental design, data analysis and verification of the microarray results have been described (Roos *et al.*, 2006). In short, mid-exponential cultures of strain 83972 grown in pooled human urine or MOPS minimal medium supplemented with 0.2% glucose were used for inoculation of 50 ml urine or MOPS to an OD₆₀₀ of 0.05 and 5 ml samples for isolation of RNA were extracted from three individual cultures at mid-exponential phase. The cultures were grown at 37°C and 130 r.p.m. Extracted samples were immediately mixed with 2 vols RNeasy Protect Bacteria Reagent (Qiagen), and incubated for 5 min at room temperature to stabilize RNA. Total RNA was isolated using the RNeasy Mini kit (Qiagen), and eluted RNA samples were treated with DNase I and repurified using RNeasy Mini Columns. The quality of the total RNA was examined by agarose gel electrophoresis and by measuring the absorbance at 260 and 280 nm. Conversion of RNA to cDNA, hybridization, washing and staining were performed according to the GeneChip Expression Analysis Technical Manual 701023 Rev. 4 (Affymetrix) and the microarrays were scanned using the GeneChip Scanner 3000. GeneChip *E. coli* Genome 2.0 Arrays (Affymetrix) were used for hybridization of the labelled cDNA. Three chips were hybridized with samples from *E. coli* 83972 grown in MOPS in triplicate and three chips were hybridized with cells grown in pooled human urine in three individual flasks. The GeneChip *E. coli* Genome 2.0 Array includes approximately 10 000 probe sets for all 20 366 genes present in the K-12 (MG1655), CFT073 (uropathogenic), O157:H7-EDL933 (enterohaemorrhagic) and O157:H7-Sakai (enterohaemorrhagic) genomes. Array normalization and expression value calculation were performed using the DNA-Chip Analyzer (dChip) 1.3 software program (<http://www.dchip.org/>) (Li & Wong, 2001). The three arrays hybridized with samples from *E. coli* 83972 grown in MOPS were used as the baseline for calculation of fold changes on the three arrays hybridized with samples from *E. coli* 83972 grown in urine.

Verification of microarray results. RT-PCR was performed to confirm DNA microarray gene expression data. Total RNA was isolated exactly as described above and treated with DNase I to remove any traces of DNA. RNA was converted to cDNA using SuperScript II (Invitrogen Life Technologies). cDNA was used directly as template for PCR and a negative control on the RNA sample (not converted to cDNA) was run in parallel to confirm that all DNA had been removed in the earlier step. The total number of cycles used in PCR ranged from 12 to 30. RT-PCR was performed to verify the transcript levels for an example gene, *papA*. The following primers were used in RT-PCR and PCR: *papA*, 620 (5'-GTGAAG-TTTGATGGGGCGACC-3') and 621 (5'-CGCAACTGCTGAGAAA-GCACC-3'); 16S, 622 (5'-CGGATTGGAGTCTGCAACTCG-3') and 623 (5'-CACAAAGTGTAAGCGCCCTC-3'). *papA* was significantly

up-regulated 19-fold in urine and showed very low signals in MOPS. *papA* could not be detected in the samples from MOPS, not even after 30 cycles of PCR, while *papA* was detected in all three urine samples, visualized as strong bands on an agarose gel. 16S was used as a normalizing internal standard and was detected with the same intensity in all samples.

Transmission electron microscopy. Cells were prepared from freshly grown colonies on LB agar and resuspended in a drop of sterile ultrapure water. A glow-discharged Formvar-coated copper grid was placed on to the drop for approximately 1 min to allow the cells to adsorb. Excess liquid was removed from the grid with a piece of filter paper before a drop of 1% ammonium molybdate (negative stain) was placed on the grid. After a few seconds the grid was blotted dry and the preparation was observed under a JEOL JEM1010 transmission electron microscope operated at 80 kV. The images were captured using an analySIS Megaview III digital camera.

RESULTS

Expression of *foc* genes in strain 83972 is up-regulated in human urine

As previously mentioned *E. coli* 83972 is an excellent colonizer of the human bladder and grows well in human urine. We speculated whether growth in human urine could affect the expression of the *foc* genes. To test this theory, DNA array analysis of gene expression was carried out. When the global gene expression profiles of strain 83972 grown in urine and MOPS medium were compared, several of the *foc* genes had altered expression; notably *focA*, encoding the major structural protein of F1C fimbriae, was highly expressed (Fig. 1). *focA* showed a high expression level in MOPS and was significantly up-regulated 1.8-fold in urine, displaying the 21st highest signal in urine out of a total of 8716 transcripts. Also the *sfaB* and *focI* genes showed

Fig. 1. Expression levels of the genes in the *foc* cluster of *E. coli* 83972 grown in MOPS and pooled human urine. The signals are calculated from biological triplicates and error bars indicate standard error; *sfaB*, *focA* and *focI* showed significantly higher levels in urine compared with MOPS. The use of *sfa* terminology (*sfaC* and *sfaB*) originates from the fact that these regulatory genes have never been characterized, but since they are almost identical to their *sfa* homologues the *sfa* terminology is used.

significant up-regulation: 2·7- and 2·4-fold, respectively. Taking these results together it seems that growth in urine up-regulates the expression of genes in the *foc* gene cluster. Arguably, expression of F1C fimbriae might be implicated in the ability of strain 83972 to colonize the human bladder.

E. coli 83972 is unable to express F1C fimbriae

F1C fimbriae can be detached from the surface of bacteria by blending and are easy to detect by Western blotting. When strain 83972 was probed for surface-associated F1C fimbriae by Western blotting with F1C-specific serum no signals could be observed, whereas the major structural component of F1C fimbriae, FocA, was readily detected in a positive control strain (Fig. 2). This indicated that strain 83972 did not produce F1C fimbriae or that these organelles were present in very low numbers.

We have previously reported that strain 83972 can produce P fimbriae, but these are non-functional (Klemm *et al.*, 2006). Since F1C and P fimbriae are morphologically identical, the *pap* genes, responsible for P fimbriae production, were deleted from the genome of strain 83972, resulting in strain 83972*pap*. Electron microscopy of 83972*pap* revealed that it is completely bald, showing no indication of either expression of F1C or any other fimbriae (Fig. 3a). Meanwhile, we proceeded to probe for the presence of FocA protein in total cell lysates of strain 83972. Indeed it turned out that although the strain did not express F1C fimbriae, small amounts of the major building element of the fimbriae, FocA, could be detected but this was apparently not assembled into fimbriae (Fig. 2).

Complementation of the *foc* genes in 83972 to identify non-functional gene products

We have previously demonstrated that structural components of type 1 fimbriae can be exchanged with similar components of F1C fimbriae (and vice versa), resulting in hybrid fimbriae (Klemm *et al.*, 1994, 1995). For example the mannose-binding FimH adhesin of type 1 fimbriae can be displayed as an integral component of F1C fimbriae, giving rise to bacterial hosts capable of yeast agglutination (Klemm *et al.*, 1994). This feature can be employed to monitor expression of F1C fimbriae in strain 83972 because, although the *fim* gene cluster in the strain has a

large deletion, the *fimH* gene is intact, expressed, and gives rise to a functional adhesin (Klemm *et al.*, 2006). When a plasmid, pPKL143, containing the intact *foc* gene cluster was transformed into strain 83972*pap* the resulting cells readily agglutinated yeast cells, indicative of surface-exposed F1C hybrid fimbriae (with integrated FimH).

To probe the *foc* gene cluster of strain 83972 for non-functional genes the strain was transformed with plasmids harbouring various *foc* genes and the resultant transformants were tested for their ability to agglutinate yeast cells (Table 2). Only one of these, transformed with plasmid pPKL160 encoding the *focCD* sector, showed positive yeast agglutination. Transformants containing a cut-down version of this plasmid (pPKL332), encoding only an intact *focD* gene, were also positive. Furthermore, fimbriae purified from the surface of 83972 cells harbouring plasmid pPKL332 reacted positively with FocA antisera in Western blotting experiments (Fig. 2). To confirm the expression of F1C fimbriae we examined the different strains by transmission electron microscopy. In contrast to cells of 83972*pap*, which were completely bald, 83972*pap* cells harbouring pPKL332 (*focD*) were highly fimbriated (Fig. 3).

Analysis of the nucleotide sequence of the *foc* gene cluster

As a parallel activity to the genetic complementation and to characterize the *foc* gene cluster in more detail, this region from 83972 was amplified by PCR and sequenced. The integrity of the sequence was evaluated by direct comparison with the equivalent genes from the genome of the sequenced UPEC strain CFT073 (Table 3). No deletions or premature stop codons were identified and the analysis revealed that all predicted open reading frames are maintained. Sequence divergence was observed for the major structural subunit-encoding gene *focA* (one base substitution) and the minor subunit-encoding gene *focF* (two base substitutions), resulting in 100 % and 98 % amino acid identity for FocA and FocF, respectively. FocF contained the two amino acid substitutions L52F and S88R as compared with CFT073. However, FocF from strain 83972 shows 99 % amino acid identity with SfaG, an S-fimbrial adhesin protein, from the uropathogenic strain 536, and the two amino acid residues in positions 52 and 88 are identical to those of SfaG in 536. Strain 536 is known to express functional S fimbriae (Ott *et al.*, 1988), indicating that the two amino acid substitutions in FocF of the 83972 relative CFT073 might not be crucial for the functionality of this subunit, which is in agreement with our yeast agglutination results (Table 2).

When the translated sequence of the *focD* gene of strain 83972 was compared with that of the similar gene in CFT073 it was found to contain two amino acid changes relative to FocD from *E. coli* CFT073: Q472L and A889V. It is interesting to note that the Q472 residue seems to be highly conserved among fimbrial ushers; BLAST analysis of FocD revealed that the residue is conserved in FocD from CFT073, Nissle 1917 and AD110, SfaF of strain 536, and FimD of

Fig. 2. Western blot analysis. Lane 1, surface proteins of *E. coli* MG1655Δ*fim*(pPKL163, all *foc* genes except *focD*); lane 2, surface proteins of *E. coli* 83972*pap*; lane 3, total cell proteins of *E. coli* 83972*pap*; lane 4, surface proteins of *E. coli* 83972*pap*(pPKL332, *focD*⁺); lane 5, surface proteins of *E. coli* 83972*pap*(pPKL143, all *foc* genes).

Fig. 3. Transmission electron micrographs of (a) *E. coli* 83972*pap*, (b) *E. coli* 83972*pap*(pPKL143, all *foc* genes), and (c) *E. coli* 83972*pap*(pPKL332, *focD*⁺). Complementation of *E. coli* 83972*pap* with a plasmid containing the *focD* gene restored its ability to produce F1C fimbriae.

O157:H7, CFT073, EDL933 and K-12. Taken together, our data demonstrate that *E. coli* 83972 is unable to synthesize F1C fimbriae because the chromosomal *focD* gene encodes a non-functional usher; however, the gene product can be complemented *in trans* by the introduction of a functional *focD* gene, leading to reinstatement of fimbrial production. The introduced version of *focD* differs from the indigenous and non-functional copy of *focD* in only two codons (i.e. Q472L and A889V). By introducing a functional copy we have essentially performed site-directed mutagenesis repair of *focD* by natural means.

DISCUSSION

The long-term bacterial occupancy of a privileged host niche such as the human bladder must involve adaptations to the host environment. It is not known how ABU strains manage to avoid challenging the host defence or escape clearance for so long in the presence of the host innate and adaptive immune systems. The adaptations that occur during the transition from symptomatic disease to asymptomatic carriage are only starting to be appreciated. ABU patients may carry a single strain for months or years, creating a condition

that resembles commensalism, but with a strain that may have evolved from a disease isolate. Several lines of evidence support the notion that the ancestor of strain 83972 was a pyelonephritic UPEC strain. First, multi-locus sequence typing (MLST) of 83972 shows that it belongs to the B2 clonal group (<http://www.mlst.net/>). *E. coli* strains belonging to group B2 are associated with pyelonephritis and other extra-intestinal invasive clinical syndromes such as bacteraemia, prostatitis and meningitis. Second, despite the fact that the *fim* and *pap* gene clusters of the strain contain mutations that destroy their function, they contain sequence information suggestive of a pathogenic past. Third, the *fimH* allele of 83972 encodes minor amino acid

Table 3. Base substitutions in the *foc* cluster of *E. coli* 83972 compared with CFT073

Gene	Base substitution	Amino acid substitution
i.r. <i>sfaCB</i> *	C154→A	
	A163→G	
	G180→A	
	C185→T	
	C192→T	
	A201→G	
<i>focA</i>	G57→A	Ala19→Ala
<i>focD</i>	A1415→T	Gln472→Leu
	T1966→C	Leu656→Leu
	C2112→T	Gly704→Gly
	C2666→T	Ala889→Val†
<i>focF</i>	C154→T	Leu52→Phe†
	A262→C	Ser88→Arg†

*i.r. *sfaCB*: intergenic region between the two regulatory encoding genes *sfaC* and *sfaB*. Position indicates nucleotide base position after *sfaC*.

†These amino acid residues are identical to those of the equivalent genes from the uropathogenic strain 536.

Table 2. Agglutination phenotype of *E. coli* 83972*pap* transformed with plasmids encoding selected *foc* genes

Plasmid	Relevant characteristics	Yeast agglutination
None		—
pPIL110-541	<i>focA</i>	—
pPKL136	<i>focC</i>	—
pPKL160	<i>focCD</i>	+
pPKL332	<i>focD</i>	+
pPKL148	<i>focFGH</i>	—
pPKL163	All <i>foc</i> genes except <i>focD</i>	—
pPKL143	All <i>foc</i> genes	+

variations (compared with the K-12 version) that are consistent with those of previously characterized pyelonephritis isolates (Sokurenko *et al.*, 1994, 1995, 2004). Finally, the strain has a copy of the F14 *papA* variant that has been associated with other virulence factors, including S and F1C fimbriae, haemolysin and cytotoxic necrotizing factor 1 from *E. coli* strains in the phylogenetic group B2 (Johnson *et al.*, 2001).

In this work we found that expression of the *foc* genes was significantly up-regulated in strain 83972 by exposure to and growth in human urine. Meanwhile, when we defined the molecular status of F1C fimbriae we found that this important fimbrial adhesin cannot be expressed due to a defunct transport system. This finding underlines the importance of F1C fimbriae in urinary tract colonization. Arguably, the ancestor of strain 83972 was once able to express F1C, type 1 and P fimbriae, all of which are associated with UPEC strains. It is interesting to note that all of these fimbriae have been demonstrated to trigger aggressive host defence mechanisms such as production of cytokines, inflammation and exfoliation of infected epithelial cells (Bäckhed *et al.*, 2002; Hedlund *et al.*, 2001; Mulvey *et al.*, 1998; Samuelsson *et al.*, 2004; Wullt *et al.*, 2003). It seems feasible that the failure of strain 83972 to trigger symptoms in a human host can to a large degree be accounted for by its inability to express functional F1C, type 1 and P fimbriae. Interestingly, it was recently found that in the UPEC strain CFT073 the loss of P and type 1 fimbriae appears to be compensated for by expression of F1C fimbriae (Snyder *et al.*, 2005). Obviously strain 83972 cannot do this because here all three systems are defunct. Also, the non-functional FocD cannot be complemented by other fimbrial ushers because the *fimD* gene is truncated (Klemm *et al.*, 2006) and complementation with the PapC usher does not work (our unpublished results). In contrast to organisms that have acquired genes for pathogenesis, *E. coli* 83972 is an example of an organism that has adapted to commensalism through gene loss and mutations. The relationship between bacterium and host in a persistent infection is a mutual trade off. In this case the bacterium has lost its primary colonization factors; however, having done so it does not damage the host and evades immune surveillance. In effect the strain has become domesticated to a degree where it does not cause any symptoms: it has become benign.

According to the literature, *E. coli* 83972 was carried by a young girl for 3 years without any symptoms. Whether the strain had already lost the ability to express F1C fimbriae previously during passage in another host or did so in this particular girl is unclear. If gene functions are directly detrimental due to conditions in the environment then mutations will be selected for which render them non-functional. In *E. coli* 83972 the F1C system is probably inactivated by adaptive mutations as a trade-off with the host. Genes of non-functional products tend to erode over time through accumulation of mutations. There are many instances where genome shrinkage has been associated with

bacterial lifestyle transition (Moran, 2002). It is interesting to note that the type 1, P and F1C fimbrial systems of *E. coli* 83972 have been incapacitated in quite different ways: the type 1 system by a major deletion encompassing 4.5 kb of the *fim* gene cluster and P fimbriae by point mutations in the *papG* gene rendering the PapG adhesin non-functional. Finally, the F1C system has been inactivated by point mutations in the fimbrial transport system. The Q472L mutation is the likely candidate for inactivation of FocD since it is highly conserved among fimbrial ushers. Arguably, this residue plays a role in the function and/or stability of the protein. However, the *foc* genes are still transcribed and the major structural protein, FocA, is produced although it is unable to reach the cell surface. This suggests that the inactivation of FocD is a recent evolutionary event.

Recently, we found that strain 83972 grows extremely well in human urine (Roos *et al.*, 2006). It might well be that these excellent growth characteristics account for its faculty for long-term bladder colonization. Strain 83972 has been used in several human inoculation studies and plays a key role in the success of this protocol by establishing bacteriuria without jeopardizing the health of the patient (Wullt *et al.*, 1998, 2000; Wullt, 2003). Deliberate colonization with *E. coli* 83972 has been shown to reduce the frequency of UTI in patients with neurogenic bladder secondary to spinal cord injury (Hull *et al.*, 2000) and the strain can prevent catheter colonization by bacterial and fungal uropathogens (Darouiche *et al.*, 2001; Trautner *et al.*, 2002, 2003). This study sheds new light on how *E. coli* 83972 has adapted to grow in the human bladder. The strain has lost the ability to express functional F1C, type 1 and P fimbriae and thus is able to persist in this environment without triggering a host immune response.

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