Urinary tract infection (UTI) is a serious health problem affecting millions of people each year. It is estimated that there are more than 10 million cases in Western Europe alone per year. The recurrence rate is high, and often the infections tend to become chronic with many episodes. UTI usually starts as a bladder infection but often evolves to encompass the kidneys and ultimately can result in renal failure or dissemination to the blood. UTI is the most common infection in patients with a chronic indwelling bladder catheter; bacteriuria is essentially unavoidable in this patient group (9). UTI is classified into disease categories according to the site of infection: cystitis (the bladder), pyelonephritis (the kidney), and bacteriuria (the blood). Colonization of urine in the absence of clinical symptoms is called asymptomatic bacteriuria (ABU). ABU occurs in up to 6% of healthy individuals and 20% of elderly individuals. ABU strains generally do not cause symptoms, and most patients with ABU do not need treatment. Furthermore, colonizing ABU strains may actually help to prevent infection by other more virulent bacteria (8, 14, 44, 45).

*Escherichia coli* is responsible for more than 80% of all UTIs and causes both ABU and symptomatic UTI (13, 39). These infections are typically caused by a single bacterial clone and are in effect monocultures. The ability of uropathogenic *E. coli* (UPEC) to cause symptomatic UTI is associated with the expression of a variety of virulence factors, including adhesins (e.g., type 1 and P fimbriae) and toxins (e.g., hemolysin) (20, 33). Bacterial adherence is generally considered to be a pivotal step in the colonization of host tissue surfaces submitted to hydrodynamic flow forces. The human urinary tract is submitted to brutal hydrodynamic shear forces, and adherence to the urinary tract epithelium enables bacteria to resist removal by urine flow. Bacterial adherence not only contributes to colonization, but also to invasion, biofilm formation, and host cell damage. The two primary fimbrial adhesins associated with UPEC strains are type 1 and P fimbriae. Type 1 fimbriae are mainly associated with cystitis and confer binding to α-d-mannosylated proteins, such as uroplakins, which are abundant in the bladder (7, 46). Expression of P fimbriae is primarily linked to pyelonephritic strains. P fimbriae recognize the α-d-galactopyranosyl-(1-4)-β-d-galactopyranoside moiety present in the globoseries of glycolipids located in the human kidney as well as on erythrocytes (21). Both type 1 and P fimbriae trigger host responses that include cytokine production, inflammation, and exfoliation of infected bladder epithelial cells (31, 36, 48).

*E. coli* strain 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 (1, 25). It is well adapted for growth in the human urinary tract, where it establishes long-term bacteriuria (1, 14, 47, 49). The strain has been used for prophylactic purposes; as such, it has been used as an alternative treatment in patients with recurrent UTI who are refractory to conventional therapy (8, 14). Here the bladders of patients are deliberately colonized with *E. coli* 83972 in order to prevent disease-causing strains from colonizing. Deliberate colonization with *E. coli* 83972 has for example been shown to reduce the fre-
quency of UTI in patients with spinal cord injury and neurogenic bladder (49), and the strain can prevent catheter colonization by bacterial and fungal uropathogens (8, 44, 45). In effect, extensive trials have shown that infection with potentially dangerous UPEC strains often does not take place in such patients as long as the ABU strain stays in the bladder. The mechanism of bladder colonization by E. coli 83972 is not known. Also, the mechanisms underlying its ability to keep other strains away are not known either. Recently, we found that E. coli 83972 is unable to express functional type 1 and P fimbriae (19). The results explained to a large degree why the strain does not cause symptoms in the host. However, important outstanding questions remain, namely, (i) how is the strain capable of efficient bladder colonization and (ii) how does the strain keep pathogenic E. coli from infecting the bladder. In this study we approach these questions.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are described in Table 1. E. coli 83972 (OR:K5:H11002) was originally isolated from a young Swedish girl (1, 25). 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, uca, and foc but does not express functional fimbrial adhesins after in vitro culture or when recovered from the urinary tract (1, 15). The E. coli strains 536, CFT073, NU14, and 1177 are all well-characterized uropathogenic strains isolated from patients with severe urinary tract infections (Table 1). Strains 536 and NU14 are naturally resistant to streptomycin. These phenotypes were used to monitor the population composition in urinary tract infections (Table 1). Strains 536 and NU14 are under study.

TABLE 1. Strains used in this study

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Relevant characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>83972</td>
<td>ABU isolate (OR:K5:H11002)</td>
<td>1</td>
</tr>
<tr>
<td>MG1655</td>
<td>K-12 reference strain</td>
<td>3</td>
</tr>
<tr>
<td>536</td>
<td>UPEC isolate (O6:K15:H31), Smr+</td>
<td>4</td>
</tr>
<tr>
<td>CFT073</td>
<td>UPEC isolate (O6:K2:H1)</td>
<td>30</td>
</tr>
<tr>
<td>PK1097</td>
<td>CFT073, fim::kan</td>
<td>This study</td>
</tr>
<tr>
<td>NU14</td>
<td>UPEC isolate (O18:K1:H7), Smr+</td>
<td>16</td>
</tr>
<tr>
<td>1177</td>
<td>UPEC isolate (O1:K1:H7)</td>
<td>28</td>
</tr>
<tr>
<td>PK625</td>
<td>1177, fim::kan</td>
<td>This study</td>
</tr>
</tbody>
</table>

Growth conditions and stabilization of RNA for microarray experiments. Human urine was collected from four healthy men and women volunteers who had no history of UTI or antibiotic use in the prior 2 months. The urine was processed after sterilized, stored at 4°C, and used the following day. Overnight cultures of E. coli 83972 were grown in pooled human urine or morphinepropanesulanic acid (MOPS) minimal medium supplemented with 0.2% glucose until reaching exponential phase and then used for inoculation of 50 ml urine or MOPS to an OD600 of 0.05. The cultures were grown at 37°C and 130 rpm, and 5-m1 samples for isolation of RNA were extracted from three individual cultures at mid-exponential phase (corresponding to an OD600 of approximately 0.4 and 0.5 in urine and MOPS, respectively), Extracted samples were immediately mixed with two volumes of RNAprotect bacteria reagent (QIAGEN AG, Basel, Switzerland), incubated for 5 min at room temperature to stabilize RNA, and centrifuged. The pellets were then stored at −80°C.

RNA isolation and cDNA labeling. Total RNA was isolated using the RNeasy Mini kit (QIAGEN AG). 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. Purified RNA was precipitated with ethanol and stored at −80°C until further use. Conversion of RNA to cDNA and microarray analysis were performed according to GeneChip Expression Analysis Technical manual 701023, rev. 4 (Affymetrix Inc., Santa Clara, CA). Briefly, 10 μg of RNA was mixed with 750 ng of random hexamer primers (Invitrogen), denatured at 70°C for 10 min, and aliquots allowed to anneal at room temperature. cDNA synthesis was performed with FirstScript II reverse transcriptase (Invitrogen). cDNA was purified using a MinElute PCR purification kit (QIAGEN) and fragmented with DNase I. Fragmented cDNA was labeled using the GeneChip cDNA labeling reagent (Affymetrix).

DNA microarray hybridization. GeneChip E. coli Genome 2.0 arrays (Affymetrix) were used for hybridization of the labeled cDNA. In total, six samples were hybridized to GeneChip E. coli Genome 2.0 arrays; 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. Hybridization, washing, and staining were performed according to GeneChip Expression Analysis technical manual 701023, rev. 4 (Affymetrix) and the microarrays were scanned using the GeneChip Scanner 3000. The GeneChip E. coli genome array contains probe sets to detect transcripts from the K-12 strain of E. coli and three pathogenic strains of E. coli. 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 (enteropathogenic), and O157:H7-Sakai (enteropathogenic) strains. Due to the high degree of similarity between the E. coli strains, whenever possible a single probe set is tiled to represent the equivalent ortholog in all four strains.

Data analysis. Array normalization and expression value calculations were performed using the DNA-Chip Analyzer (dChip) 1.3 software program (http://www.dchip.org/) (23). The invariant set normalization method (24) was used to normalize arrays at probe cell level to make them comparable, and the model-based (perfect match/mismatch) method was used for probe selection and com-
putative colony counts. Urine was collected from each mouse at 24 h after inoculation for quantification. The catheter was removed immediately after the challenge, and mice were returned to their cages. India ink (for visualization of inocula at autopsy), was instilled directly into the bladder using a 1-ml tuberculin syringe attached to the catheter. The catheter was inserted into the bladder after sterile swabbing with 10% povidone-iodine solution, which was removed with sterile water. Mice were catheterized using a sterile Teflon catheter (0.28-mm internal diameter, 0.61-mm outer diameter, and 25-mm length; Terumo) by inserting the device directly into the bladder through the urethra. An inoculum of 25 CFU of bacteria was used for this study (12). Female CBA mice (8 to 10 weeks) were purchased from the Animal Resources Center, Western Australia, and housed in sterile cages with ad libitum access to sterile water. Urine was collected from each mouse 24 h prior to challenge and examined microscopically with a hemocytometer and by culture. The following primers were used in RT-PCR and PCR: popA, 620 (5'-GTGAA GTTTGATGGGGCCGACC) and 621 (5'-CGCAACTGCTGAGAAAGCAC); papA, 620 (5'-GGATTGGAGTCTGCAACTCG) and 621 (5'-CGCAACTGCTGAGAAAGCACC); and 16S, 622 (5'-CGGATTTGAGCTGCAACTCG) and 623 (5'-CACAAGGTTGAAAGCCTC).

Murine model of UTI. A modification of the murine model of UTI was used for this study (12). Female CBA mice (5 to 10 weeks) were purchased from the Animal Resources Center, Western Australia, and housed in sterile cages with ad libitum access to sterile water. Urine was collected from each mouse 24 h prior to challenge and examined microscopically with a hemocytometer and by culture. The following primers were used in RT-PCR and PCR: popA, 620 (5'-GTGAA GTTTGATGGGGCCGACC) and 621 (5'-CGCAACTGCTGAGAAAGCAC); papA, 620 (5'-GGATTGGAGTCTGCAACTCG) and 621 (5'-CGCAACTGCTGAGAAAGCACC); and 16S, 622 (5'-CGGATTTGAGCTGCAACTCG) and 623 (5'-CACAAGGTGGGATTGGAGTCTGCAACTCG).

RT-PCR. Reverse transcription-PCR (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 products were examined by agarose gel electrophoresis. The following primers were used in RT-PCR and PCR: popA, 620 (5'-GTGAA GTTTGATGGGGCCGACC) and 621 (5'-CGCAACTGCTGAGAAAGCAC); papA, 620 (5'-GGATTGGAGTCTGCAACTCG) and 621 (5'-CGCAACTGCTGAGAAAGCACC); and 16S, 622 (5'-CGGATTTGAGCTGCAACTCG) and 623 (5'-CACAAGGTGGGATTGGAGTCTGCAACTCG).

RESULTS

Growth characteristics of E. coli 83972. In an attempt to understand the bladder colonization properties of E. coli strain 83972, we initially focused on its growth characteristics in human urine. Under normal conditions, an adult human being produces 1 to 2 liters of urine per day, all of which pass through the bladder. Arguably, the ability to grow in human urine must be an important criterion for colonization of the bladder. The strain grows well in human urine in vitro and, depending on the individual batch of urine used, grows exponentially, with doubling times ranging from 45 min to 60 min, and it levels out in stationary phase with an OD₆₀₀ of 0.5 to 0.9. The E. coli K-12 reference strain MG1655 is unable to grow in urine and cannot be used for comparison. However, in LB medium strain 83972 shows slightly better growth characteristics than the K-12 strain (data not shown). For comparison, we probed the growth characteristics of some well-characterized UPEC strains, viz. E. coli strains 536, CFT073, NU14, and 1177, all isolated from severe cases of urinary tract infections (Table 1). Surprisingly, all four UPEC strains performed significantly poorer than strain 83972 when grown in urine, with doubling times in the exponential phase 10 to 100% longer (two-sample two-tailed t test, P < 0.002), and they entered stationary phase at an OD₆₀₀ of 10 to 30% lower than that of 83972 (P < 0.04) (Fig. 1). Accordingly, due to its faster doubling time and higher carrying capacity in urine, it is highly likely that strain 83972 should be able to outcompete these UPEC strains within a relatively modest number of generations.

E. coli 83972 competition with UPEC strains in human urine. In order to monitor if the superior growth characteristics of strain 83972 were a general phenomenon, we competed it against our panel of well-characterized UPEC strains (Table 1). This was done by incubation of sterile human urine with equal amounts of strain 83972 and the UPEC strain in question.
and then monitoring the percentage of each strain after overnight cultivation by selective plating. In all cases, strain 83972 outcompeted the UPEC strain; even though the UPEC strain constituted 50% of the population at the start of the cultivation, it ended up constituting only 3% to 4% in the case of CFT073, NU14, and 1177 and 29% in the case of strain 536 (paired two-tailed t test, \( P < 0.001 \)) (Fig. 2). Figure 1C, displaying the competition between 83972 and NU14 during the first 7 hours, reveals that \( E. coli \) 83972 constitutes about 80% of the population already after 2 h. To further investigate the degree of advantage by \( E. coli \) 83972, it was competed against NU14 with a starting ratio of 1 to 20; although NU14 constituted 95% of the population at the start of the experiment, it accounted for only 7% of the population after 17 h (paired two-tailed t test, \( P < 0.01 \)). Taken together the data show that strain 83972 is able to outgrow a representative spectrum of UPEC strains.

\section*{Global gene expression of \( E. coli \) 83972 in human urine.}

The genomic expression profile of \( E. coli \) 83972 grown in pooled, filtered human urine was compared with the strain grown in MOPS-glucose medium. Samples were extracted during mid-exponential growth phase in order to best mimic the situation the cells encounter in the human bladder; in the bladder any colonizing organism will continuously be provided with fresh nutrients. RNA isolated from exponentially growing cultures (in triplicate) was used for hybridization of microarrays, and the data were analyzed as described in Materials and Methods. For analysis, the GeneChip \( E. coli \) Genome 2.0 array was employed. This microarray contains \( \sim 10,000 \) probe sets for all 20,366 genes present in \( E. coli \) strains MG1655, CFT073, EDL933, and O157:H7-Sakai.

The analysis of the microarray data is described in Materials and Methods. Whether a fold change in expression of a gene observed between two different arrays could be considered significant or not was not solely dependent on the magnitude of the change, but also on the absolute signal on the two arrays. A large up-regulation of a gene has to have a signal that is present on the sample array, but not on the baseline array, while in the case of a small up-regulation (low fold change, closer to 1.0), the signal has to be present and high on both the sample and the baseline array, and vice versa for down-regulated genes. Therefore, in our analysis we have only considered an up- or down-regulation to be significant if it meets these criteria, i.e., for a low fold change (1.4 to 1.9) to be considered significant the signal has to be present and high on all three baseline arrays as well as the three sample arrays. Moreover, the comparison criteria were carefully chosen to make sure that the empirical FDR was kept low (0.3%, i.e., seven false-positive genes). The estimation of FDR has become widely accepted as appropriate (10) and, furthermore, it has been argued that FDR is a more natural scale to work on rather than the \( P \) value (34).

Overall, 626 genes (10%) were expressed at significantly higher levels in human urine and 1,646 genes (25%) were significantly down-regulated during growth in urine, whereof 383 and 1,039 genes, respectively, were MG1655 transcripts and the remaining genes were CFT073 (uropathogenic) transcripts. Classification of the MG1655 genes into functional groups (41, 42) revealed that a large number of the genes involved in carbohydrate transport and metabolism, energy production and conversion, and inorganic ion transport and metabolism were significantly up-regulated in urine (Table 2). Some of the genes belonging to these groups can also be found among the genes displaying the highest fold changes in urine compared with minimal medium, particularly genes encoding proteins involved in iron transport and metabolism (Table 3).

\section*{Iron acquisition genes.}

The concentration of soluble forms of iron is very low in the urinary tract and is a growth-limiting factor for bacteria. Consequently, iron acquisition plays an important role in bacterial survival and pathogenicity. In strain 83972 many genes involved in iron uptake and transport were significantly up-regulated in human urine (Fig. 3A). Transcription from genes encoding proteins involved in enterobactin synthesis, \( entBCDEF \), were up-regulated 20- to 44-fold, and the gene encoding the highly specific ferric enterobactin receptor, \( fepA \), revealed one of the highest signals and was up-regulated 51-fold. Genes encoding FhuA, FhuE, and IutA, which transport hydroxamate siderophores, were induced 13-, 12-, and 38-fold, and genes encoding Fiu and Ctr, which transport catecholate siderophores, were induced 17- and 21-fold. The transport of iron across the outer membrane is dependent upon the energy-transducing TonB-ExbB-ExbD complex; these genes were up-regulated between 12- and 18-fold in urine.

Genes encoding proteins responsible for iron uptake without siderophores, \( sitABCD \), were significantly up-regulated (17- to 47-fold); \( sitB \) showed the 10th highest signal in urine. It has been demonstrated that the \( sit \) cluster is required for full virulence of \( Salmonella enterica \) serovar Typhimurium (17). Other virulence-associated genes, \( iutA \) and \( iucABCD \), responsible for aerobactin uptake and synthesis, were up-regulated 29- to 48-fold in urine. The \( iro\) genes, encoding a siderophore receptor that contributes to urovirulence (35), was highly up-regulated in urine (37-fold), as were the rest of the genes in the \( iro \) cluster (\( iroBCDE \) were induced 6.3- to 76-fold). The genes encoding the Chu heme transport system, \( chuA\) were up-regulated 5.5- to 46-fold; \( chuA \) has been shown to be im-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Results from growth competition experiments (1:1) between \( E. coli \) 83972 and UPEC strains after 17 h of growth in human urine. Each value is an average of four individual shake flasks obtained from two separate experiments in different batches of urine. In all four competition experiments, strain 83972 was present in significantly higher numbers than the UPEC isolate (paired two-tailed \( t \) test, \( P < 0.001 \)).}
\end{figure}
important for the virulence of a uropathogenic *E. coli* strain (43). The small regulatory antisense RNA (*ryhB*) involved in iron homeostasis was up-regulated 38-fold. *RyhB* down-regulates the mRNA levels for the genes that are known to be positively regulated by Fur (ferric uptake regulator) (29). The *fur* gene itself showed no change in expression in urine compared with MOPS. Taken together, the data indicate that strain 83972 has numerous genes encoding products that are well suited to cope with an iron-poor environment and that these are highly expressed during growth in urine.

**Carbohydrate transport and metabolism.** The *ara* cluster belonged to one of the most up-regulated in urine (Table 2). The genes *araABD*, which are responsible for the conversion of arabinose to xylulose-5-phosphate, were up-regulated 32-, 46-, and 46-fold, respectively. The genes encoding arabinose transporters, both the arabinose/proton symporter gene *araE* and...
the ABC transporter encoding genes araFGH, were up-regulated (40-, 14-, 17-, and 25-fold, respectively).

The degradation of sugar acids was highly up-regulated in urine. All the genes involved in the degradation pathways of galacturonate, glucuronate, and galactonate were significantly up-regulated up to 77-fold, and the genes encoding transporters of these compounds were up-regulated up to 44-fold (Fig. 4). The fructuronic acid transporter-encoding gene, gntP, which is divergently transcribed from uxuAB, was up-regulated 4.2-fold. The gene encoding N-acetylneuraminic lyase, nanA, which catalyzes the breakdown of sialic acid, was up-regulated 19-fold. Furthermore, many genes involved in the transport of carbohydrates were up-regulated in urine compared with minimal medium; genes encoding proteins responsible for transport of sorbitol (srlAB), galactose (mglABC), maltose (lamB), xylose (syfFGH), and mannose/fructose (manXYZ) were all significantly up-regulated 2.4- to 34-fold.

**Adhesins.** *E. coli* 83972 harbors a nonfunctional pap gene cluster, encoding P fimbriae, and a truncated fim gene cluster, encoding type 1 fimbriae (19). Our microarray data revealed that half of the pap genes were significantly up-regulated in urine: papHDFJ were up-regulated 2- to 5-fold, and the gene encoding the major subunit, papA, was up-regulated 19-fold (Fig. 3B). While the signals for fimEAI could not be detected in *E. coli* 83972 due to a large deletion in the fim cluster, the remaining fim genes, fimBDFGH, displayed low signals and were all down-regulated in urine.

The foc/sfa cluster encoding F1C fimbriae was partly up-regulated in *E. coli* 83972, with focA, encoding the major subunit, up-regulated 1.8-fold in human urine. Other fimbrial genes significantly changed in urine belonged to the recently characterized auf cluster (6), for which genes were down-regulated 4.3- to 221-fold. Furthermore, the hypothetical fimbrilike protein precursors yadN and ygiL were down-regulated 4.3- and 115-fold, respectively, and two genes encoding putative adhesins, eaeH and c4424, were down-regulated 12- and 3.8-fold. Expression from the flu gene encoding aggregation factor Ag43 was very low and showed no significant change when grown in urine.

**Other virulence factors.** The pore-forming hemolysin (HlyA) is considered an important virulence factor in *E. coli* extraintestinal infections, such as those of the upper urinary tract. The genes determining the synthesis, activation, and transport of hemolysin, hlyCABD, were all down-regulated in urine (106-, 9.2-, 2.8-, and 90-fold, respectively). This was confirmed by lack of hemolysis on blood agar plates (data not shown). Of the genes encoding proteins involved in biosynthesis of lipopolysaccharides, most were significantly down-regulated in urine, the genes involved in the enterobacterial common antigen biosynthesis pathway (rfaACDGHMT, wecBF, and
rfe) were all down-regulated 2.0- to 6.3-fold, the genes involved in colonic acid biosynthesis (cpsB, gmd, fel, and ugd) were down-regulated 4.0- to 26-fold, and the genes involved in the synthesis of lipid A (lpxBHLP, kdsABC, htrB, and kdtA) were down-regulated 1.6- to 6.0-fold. The rcsA gene, encoding a positive regulator of capsular polysaccharide synthesis, was down-regulated 16-fold in urine. The genes kpsEDCS, involved in the transport of polysaccharide to the cell surface, were all down-regulated 2.5- to 6.5-fold. The rfaH gene is another virulence-associated gene which displayed very low signals and significant down-regulation in urine (3.7-fold). RfaH is a global regulator which modifies expression of several virulence factors, and disruption of the rfaH gene in uropathogenic E. coli has been shown to result in a significant decrease in virulence (32).

Taken together the array data show that E. coli 83972 expresses all known iron transport and uptake systems when grown in urine; the strain also showed high expression levels of genes involved in uptake and metabolism of nutrients found in urine, while most of the genes encoding known virulence factors were down-regulated in urine. It is obvious that the strategy to keep and express growth-enhancing genes while shutting off virulence genes that may provoke the host response must be an optimal approach for successful long-term colonization of the urinary tract.

Verification of microarray results. RT-PCR was performed to verify the transcript levels for an example gene, papA. 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.

The expression levels of the fim genes revealed the sensitivity of the microarrays. Sequencing of the fim cluster of E. coli 83972 revealed that a large part of the cluster is deleted in the strain, i.e., a 4.25-kb deletion between fimB and fimD, resulting in complete absence of fimEAIC, leaving only fimF, fimG, and fimH unaffected (19). The signal from fimEAIC was very low and varied between 7 and 53, with an average of 27 and 35 for MOPS and urine, respectively. The signals of fimBDGFH varied between 136 and 1,209 with an average of 911 and 275 for MOPS and urine, respectively. The signal levels of the different fim genes on the arrays correspond well to the actual presence of fim genes in E. coli 83972.

E. coli 83972 competition with UPEC strain NU14 in mouse bladder. Our growth studies combined with the global gene expression data demonstrate that E. coli 83972 is highly adapted to growth in human urine. Since other studies have demonstrated a prophylactic effect of this strain in the presence of other pathogens, we competed 83972 against UPEC NU14 in the mouse UTI model. Mice were infected with equal numbers of NU14 (alone) or NU14 and 83972 (in combination, 50% mixed infection of each). After 24 h, the total number of bacteria in the urine of mice infected with NU14 or an equal combination of NU14 and 83972 was almost identical (Fig. 5).

However, when examining the percentage of each individual strain in the mice receiving the mixed combination of strains, we observed that 83972 was almost totally dominant (98.7% versus 1.3%; paired two-tailed t test, P < 0.001). Thus, 83972 is able to outcompete UPEC in an in vivo UTI infection model in addition to in vitro growth experiments in human urine.

DISCUSSION

Healthy adult humans normally produce 1 to 2 liters of urine per day, which must pass through the bladder, corresponding...
to average flow rates of 40 to 80 ml per hour. An adult human bladder has a volume of 200 to 400 ml, and micturition causes the release of roughly the same volume of urine; the volume of urine remaining in the bladder following micturition is ~1 ml (38). The contribution of bladder hydrodynamics on the elimination of bacteria has been recognized for several decades (5, 27), and it has been suggested that without adhesin-assisted attachment to the bladder surface, *E. coli* would not be able to overcome the losses caused by micturition and, therefore, it would be unable to establish in the urinary tract (2, 40). Implicit in this suggestion is the notion that the growth rate of *E. coli* in urine is too slow to cope with the losses incurred by micturition. Meanwhile, mathematical modeling suggests that if the growth rate of a strain is high enough it will be able to establish in the bladder in an adhesion-independent manner. Indeed, a theoretical analysis of bacterial growth in the bladder suggested that many urinary tract isolates of *E. coli* had growth rates that were fully compatible with nonadhesive establishment in the bladder (11). So far, no studies have been published that show that a high growth rate of a UPEC strain can permit colonization of the urinary tract independent of specific adhesion. Recently, Jarboe et al. predicted that *pap* expression was inversely related to growth rate, indicating that at high growth rates adhesion may be unnecessary for persistence, and so a decrease in fimbral expression at high growth rates potentially conserves cellular resources without decreasing the probability of survival (18). A study on the UPEC isolate NU14 revealed that antibiotic resistance mutations led to significant reduction of biological fitness (defined as decrease in growth rate), providing a possible explanation for the observed negative correlation between fluoroquinolone resistance and bacterial virulence; the study revealed that the loss of fitness could potentially conserve cellular resources without decreasing the probability of survival (18).

*E. coli* strain 83972 was recently demonstrated to be unable to express functional versions of type 1 and P fimbriae, the two fimbriae types that are recognized as being the primary adhesins of UTI *E. coli* strains (19). Furthermore, the strain has never been reported to adhere to any kind of cells originating from the human urinary tract (1, 15, 47). The lack of adhesion could to a large degree account for the inability to cause symptoms in the human host. However, it raises the issue of how the strain is capable of staying in the bladder and how it seems to be able to prevent bladder colonization by pathogenic *E. coli*. The results presented herein indicate that strain 83972 grows very well in human urine in vitro, with doubling times generally being 45 to 60 min. In some experiments, even shorter doubling times were observed. Indeed, a comparison of the doubling time of the 83972 strain with those of a spectrum of well-characterized UPEC strains showed that it grows faster than any of them. Also, it grows to higher maximum cell densities and exhibits a shorter lag phase than any of the UPEC strains investigated. This enhanced growth capacity in urine was not a unique property of 83972, since other ABU isolates from our laboratory strain collection exhibited similar growth characteristics (data not shown).

When *E. coli* 83972 was pitted against the UPEC strains in pairwise competition experiments, it outcompeted all of them to a significant degree, i.e., the ABU strain constituted 71 to 97% of the population after overnight growth. Mathematical modeling of the competition experiments, using the observed doubling times (Fig. 1) and assuming exponential growth, resulted in numbers closely resembling the values obtained experimentally (Fig. 2). These results were confirmed in mouse experiments; the 83972 strain was almost totally dominant in the urine of mice 24 h after receiving a mixed challenge consisting of 83972 and the UPEC isolate NU14 (1:1). *E. coli* 83972 can establish long-term bacteriuria in humans (1, 14, 47, 49) and has been used extensively for treatment of patients with recurrent UTI to avoid the establishment of disease-causing strains (8, 14, 44, 45, 49). Based on the results presented here, we suggest that the ability of *E. coli* 83972 to establish in the human bladder as well as its prophylactic properties against UPEC strains is first and foremost due to its excellent growth properties in human urine. In theory, strain 83972 could also compete with other strains by killing them. However, we have tested the strain for colicin production and it proved negative in this faculty.

According to the literature, *E. coli* 83972 had grown in the bladder of a girl with ABU for at least 3 years (1). This represents a substantial number of generations, i.e., more than 30,000, assuming generation times comparable to those observed in the present study. During this period of time the strain must have adapted considerably to this particular environmental niche. The strain lost the ability to express functional type 1 and P fimbriae probably as an evolutionary trade-off with the host defense. This ensured that it did not attract the attention of aggressive host defense mechanisms, such as cytokine production, inflammation, and exfoliation of infected bladder cells. However, in order to avoid being flushed out of the system, it had to adapt to the growth medium, i.e., human urine, and to optimize its growth rate to keep pace with the flow rate in the bladder. Whether this happened during the three-plus years it was carried by the particular girl or before then in other hosts is not possible to conclude. It is, however, interesting that the girl suffered from a voiding problem and was unable to empty her bladder completely, thus leaving a considerable volume of residual urine (1, 25). Arguably, this would provide the 83972 strain with an ideal “training” environment for optimizing its ability to grow in urine.

In a previous study on bacterial growth adaptation to a specific growth medium, it was shown that after 10,000 generations *E. coli* strains had increased in fitness by ~50% for growth on glucose medium (22). In line with this notion, strain 83972 must have accrued genetic changes that have favored its fitness for growth in urine. Human urine is a very complex growth medium, and the composition of urine fluctuates daily and varies with the person and diet. It is, however, known that iron availability is a limiting factor. Our array data indicated that strain 83972 has adapted well to growth in this iron-limiting environment, significantly increasing the expression of the majority of all known genes involved in iron uptake and transport when grown in urine compared with minimal medium. Furthermore, the array data revealed high expression levels of genes involved in transportation and degradation pathways of sugar acids and carbohydrates, indicating how *E. coli* 83972 is able to reach high growth rates in human urine by efficiently utilizing the nutrients available in this growth medium. It should be noted, however, that even though the *E. coli* arrays employed in this study include transcripts from the uropathogenic strain CFT073, the whole genome of *E. coli* 83972
has not yet been sequenced and characterized and, therefore, the possibility that strain 83972 carries unique genes enabling a faster growth in urine cannot be ruled out. It remains to be seen whether the mechanisms utilized by E. coli 83972 are superior to those of UPEC strains or whether this strain has evolved additional, yet-undescribed mechanisms for iron sequestration and growth. We are currently examining these possibilities further.

ACKNOWLEDGMENTS

We thank Birthe Jul Jørgensen for expert technical assistance and Hugh Connell for helpful discussions regarding the mouse UTI model. This work was supported by grants from the Danish Medical Research Council (22-03-0462), the Danish Research Agency (2052-03-0013), the Australian National Health and Medical Research Council (301163 and 401714), and the University of Queensland.

REFERENCES


Editor: J. B. Bliska