Molecular Characterization of the *Escherichia coli* Asymptomatic Bacteriuria Strain 83972: the Taming of a Pathogen

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*Escherichia coli* 83972 is a clinical asymptomatic bacteriuric isolate that is able to colonize the human urinary bladder without inducing an immune response. Here we demonstrate that one of the mechanisms by which this strain has become attenuated is through the mutation of its genes encoding type 1 and P fimbriae.

Urinary tract infections (UTI) are among the most common infectious diseases of humans and a major cause of morbidity and mortality. Acute pyelonephritis and asymptomatic bacteriuria (ABU) represent the two extremes of UTI. Acute pyelonephritis is a severe systemic infection caused by uropathogenic *Escherichia coli* (UPEC) (6, 7, 29, 31). ABU, on the other hand, is an asymptomatic carrier state in which patients may carry $>10^5$ CFU/ml of a single *E. coli* strain for years without provoking a host response. In early studies, this was explained by a lack of virulence genes; however, molecular epidemiology has shown that $>60\%$ of ABU strains carry virulence genes but fail to express the phenotype (18, 19).

The ability of UPEC to cause symptomatic UTI is enhanced by adhesins, including type 1 and P fimbriae (11, 17). P fimbriae enhance the establishment of bacteriuria and activate the innate immune response in animal models and in the human urinary tract (2, 20, 21, 33, 35, 36). Binding is mediated by the PapG adhesin, which is located at the tips of the fimbriae and which recognizes the $\alpha$-d-galacto-pyranoside receptor epitope in the globoseries of glycolipids (3, 12, 13). Type 1 fimbriae enhance colonization, induce host responses in the murine UTI model, and promote biofilm formation and invasion (4, 14, 16, 23). Receptor binding is also mediated by an adhesin located at the tips of the fimbriae (FimH) that binds to $\alpha$-d-mannosylated proteins, such as uroplakins, which are abundant in the bladder (32). In this study, we characterized the type 1- and P-encoding fimbrial genes from the prototypical ABU strain *E. coli* 83972. The strain is a clinical isolate capable of long-term bladder colonization (1). It was isolated from a patient with ABU who had carried it for 3 years, and it has been used in colonization studies as a prophylactic agent to prevent UTI in humans (2, 8, 30, 34, 35).

*E. coli* 83972 does not express a detectable type 1 fimbral phenotype when recovered from the urinary tract or after in vitro subculture (35). However, previous genetic analysis of the strain revealed that it contains the genes for type 1 fimbriae (9). To examine the *E. coli* 83972 type 1 fimbra-encoding genes in more detail, we performed a series of Southern hybridizations with probes spanning different regions within the MG1655 type 1 fimbrial gene cluster. A positive hybridization signal was obtained with a fimH gene probe but not with fimE or fimAIC probes (Fig. 1). Subsequent PCR amplification and sequencing of the fim cluster from *E. coli* 83972 revealed a 4.25-kb deletion affecting all genes except those encoding the minor components fimF, fimG, and fimH (Fig. 1). The chromosomally located fimH gene was expressed as a functional product, since the transformation of *E. coli* 83972 with plasmid pPKL114 (containing fimBEACDFG) induced a mannose-sensitive agglutination of yeast cells (Table 1). Sequencing of the fimH gene from *E. coli* 83972 revealed the following changes relative to FimH from *E. coli* K-12: V48A, G87S, N91S, and S99N.

*E. coli* 83972 reportedly contains pap gene sequences (9) but has never been shown to express P fimbriae. However, when we grew 83972 on a solid medium and examined the cells by transmission electron microscopy, we observed that the majority produced fimbriae (Fig. 2A). Interestingly, very few cells produced fimbriae when grown as liquid cultures. Purification and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the fimbriae revealed a prominent 18-kDa protein that was confirmed by N-terminal amino acid sequencing to be the PapA major subunit (Fig. 2B). *E. coli* 83972 cells expressing these P fimbriae did not hemagglutinate human red blood cells (RBCs), bind to human uroepithelial cells, or bind to Galα1-4Galβ-containing glycolipids (data not shown). Thus, strain 83972 produces P fimbriae that are unable to bind to any known receptor targets.

The pap gene cluster of *E. coli* 83972 was amplified by PCR and sequenced. A comparison of the amino acid sequence deduced from each gene with the equivalent genes from UPEC CFT073 revealed that the greatest divergence occurred in papA, papE, and papG (Fig. 2E). The function of the 83972 PapG adhesin was assessed by complementation with the following plasmids: (i) pDD3 (all pap genes from UPEC J96 except papG) and (ii) pDD4 (papG from UPEC J96). Only *E. coli* 83972 (pDD4) cells readily agglutinated RBCs and bound to human uroepithelial cells (Fig. 3A and B; Table 1). Thus, the recognition of receptor targets by the P fimbriae of *E. coli* 83972 can be complemented in trans by a plasmid carrying a...
functional papG gene. Western blot analysis of fimbrial proteins using PapG-specific polyclonal antisera revealed that PapG is expressed and suggests that it is incorporated into the fimbrial structure (Fig. 2C). This interpretation is supported by the fact that E. coli 83972 produces fimbriae of normal length and morphology (previous studies of fimbrial biogenesis have demonstrated that disruption of the adhesin-encoding gene results in the synthesis of organelles of aberrant length and morphology) (24). It is possible that the lack of function of PapG may be associated with minor amino acid changes in the protein sequence. In this respect, all but one of the residues predicted to contribute to the PapG binding site (5) are conserved in the E. coli 83972 PapG sequence (Fig. 4). By analogy with the FimH adhesin (22, 25), variations that alter the con-

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<th>Plasmid</th>
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<td>Yeast cells</td>
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<td>None</td>
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<tr>
<td>pPKL4 (all fim genes)</td>
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<td>pPKL114 (all fim genes except fimH)</td>
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<td>pPAPS (all pap genes from J96)</td>
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<td>pDD4 (papG only)</td>
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*–*, negative; +, positive.
formational stability of the protein loops that carry the receptor-interacting residues may also account for its lack of function.

_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 P and type 1 fimbriae previously during passage through another host or did so in this particular girl is unclear. However, several lines of evidence support the notion that the ancestor of _E. coli_ 83972 was a pyelonephritic UPEC strain: (i) the FimH allele of 83972 contains minor amino acid variations that are consistent with those of previously characterized pyelonephritis strains (26–28); (ii) the strain is able to express P fimbriae, albeit an apparently nonadhesive type; (iii) multilocus sequence typing of 83972 shows that it belongs to the B2 clonal group (http://www.mlst.net/) and this group contains _E. coli_ strains associated with pyelonephritis and other extraintestinal invasive clinical syndromes such as bacteremia, prostatitis, and meningitis; and (iv) the strain possesses the F14 PapA allele, which has been associated with other virulence factors, including S and F1C fimbriae, hemolysin, and cytotoxic necrotizing factor 1 from _E. coli_ B2 strains (10). Genes of nonfunctional products tend to erode over time through accumulation of mutations, and there are many instances where genome shrinkage has been associated with bacterial lifestyle transition (15). In _E. coli_ 83972, the two primary adhesive organelles associated with uropathogenesis have been inactivated by adaptive mutations as a trade-off with the host.
The characterization of the fim- and pap-encoding genes in this study illustrates an important issue with regard to the current molecular knowledge of *E. coli* 83972. Previous studies demonstrating the presence of these genes in *E. coli* 83972 failed to correlate with its phenotypic characteristics (9, 33). Here we have shown for the first time that *E. coli* 83972 contains only some of the type 1 fimbrial genes and is not capable of producing these organelles. The finding that *fimH* is functional and constitutively expressed may explain a previous report that identified a clone capable of mannose-sensitive hemagglutination from a recombinant cosmid library derived from 83972 (9). In the case of P fimbriae, these organelles are expressed, but their function remains unknown since they do not bind to defined receptor targets. 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 P and type 1 fimbriae and is thus able to persist in this environment without triggering a host immune response. 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 mutation.

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**REFERENCES**


Specificity of binding of a strain of uropathogenic Escherichia coli to Gal

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