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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 asymptomatia 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 α-D-galatopyranosyl-(1-4)-β-D-galactopyranoside 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 α-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 fimbrial 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 fimbria-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 *fimBEAICDFG*) 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

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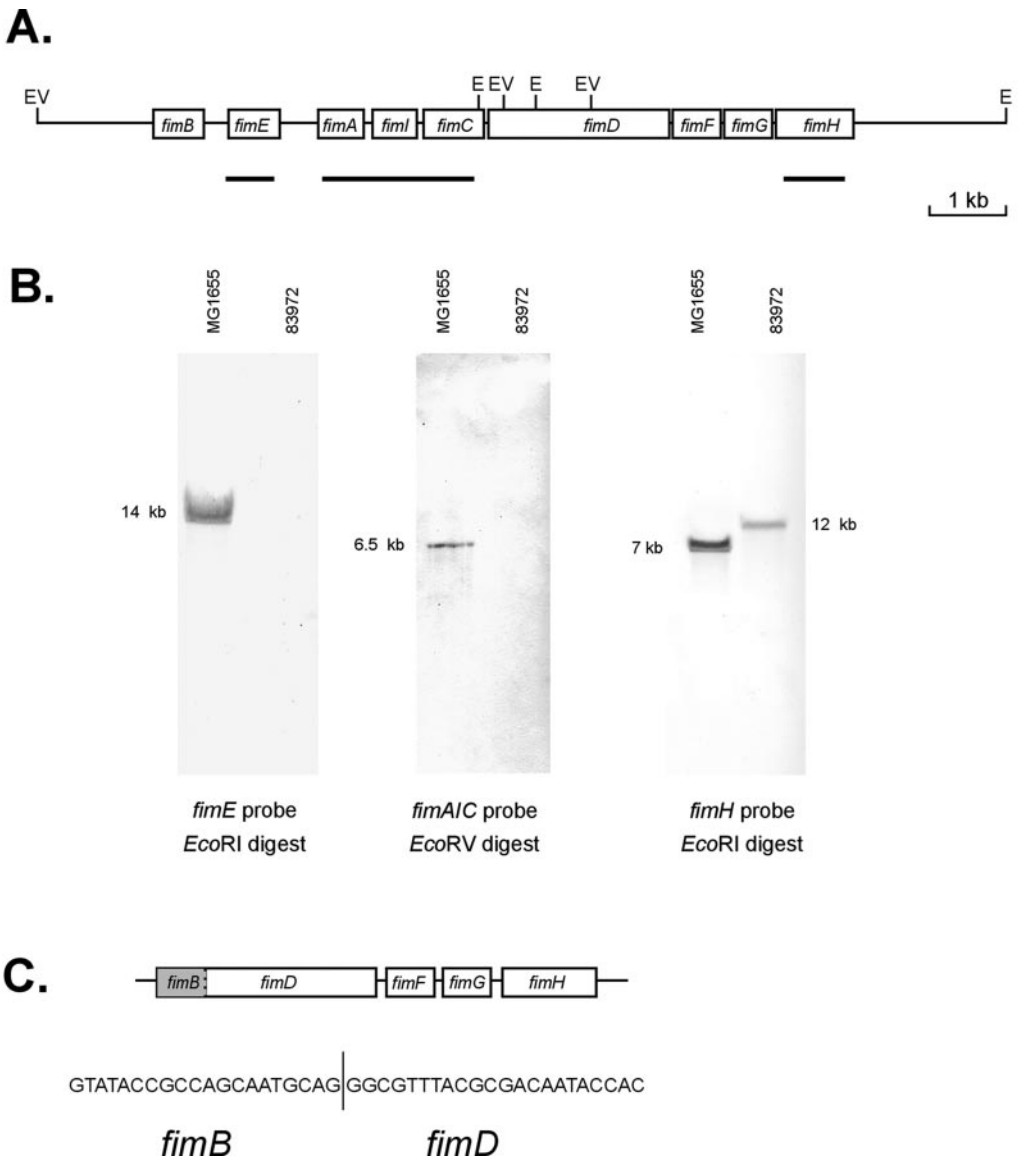

FIG. 1. (A) Physical map of the *fim* gene cluster from *E. coli* MG1655 indicating the arrangement of the genes, the position of relevant restriction enzyme sites (E, EcoRI; EV, EcoRV), and the region covered by each of the probes. (B) Southern blot analysis of total genomic DNA from *E. coli* MG1655 and 83972 digested to completion with either EcoRI or EcoRV and probed with the *fimE* gene, the *fimA*, *fimB*, and *fimC* genes, and the *fimH* gene (as indicated). The sizes of the hybridizing fragments are indicated in kilobase pairs. (C) Structure of the *fim* locus in *E. coli* 83972 indicating the precise start and end points of the deletion between *fimB* and *fimD*.

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-

TABLE 1. Agglutination profile of LB agar-grown *E. coli* 83972 containing plasmid-borne encoded fimbrial genes

Plasmid	Agglutination of[a]:	
	Yeast cells	Human type A RBC
None	−	−
pPKL4 (all *fim* genes)	+	−
pPKL114 (all *fim* genes except *fimH*)	+	−
pPAP5 (all *pap* genes from J96)	−	+
pDD3 (all *pap* genes except *papG*)	−	−
pDD4 (*papG* only)	−	+

[a] −, negative; +, positive.

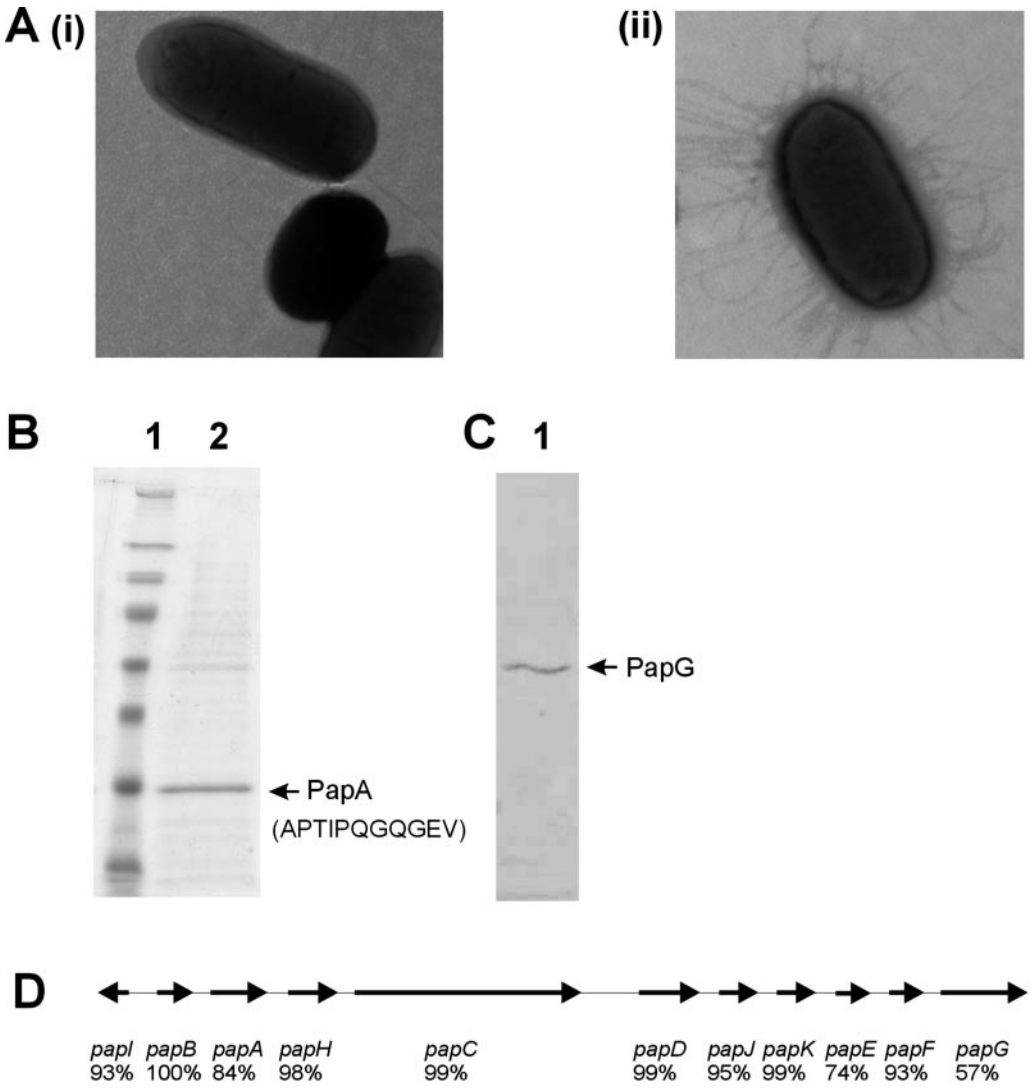

FIG. 2. (A) Transmission electron micrographs of *E. coli* 83972 grown (i) in LB broth and (ii) on LB agar. Almost all cells harvested after growth on agar produced P fimbriae, while less than 5% of cells grown as liquid cultures produced any fimbriae. This may explain previous observations that *E. coli* 83972 does not produce fimbriae. (B) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of purified fimbrial proteins from *E. coli* 83972 (lane 2). The N-terminal amino acid sequence of the PapA fimbrial protein is indicated in brackets. Molecular size markers (lane 1) are 250, 98, 64, 50, 36, 30, 16, and 6 kDa. (C) Corresponding Western blot analysis of the same purified fimbrial preparation using a PapG-specific polyclonal antiserum indicating the expression of the PapG adhesin. (D) Physical map of the *pap* gene cluster from *E. coli* 83972 indicating the arrangement of the genes and the amino acid identity of each of the predicted proteins with the respective protein from *E. coli* CFT073. The predicted translational product of the *papA* gene is identical to the first 10 residues identified by N-terminal sequencing. PapA belongs to the F14 allele group, while PapG belongs to the class III allele group (nucleotide accession number, DQ010312).

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.

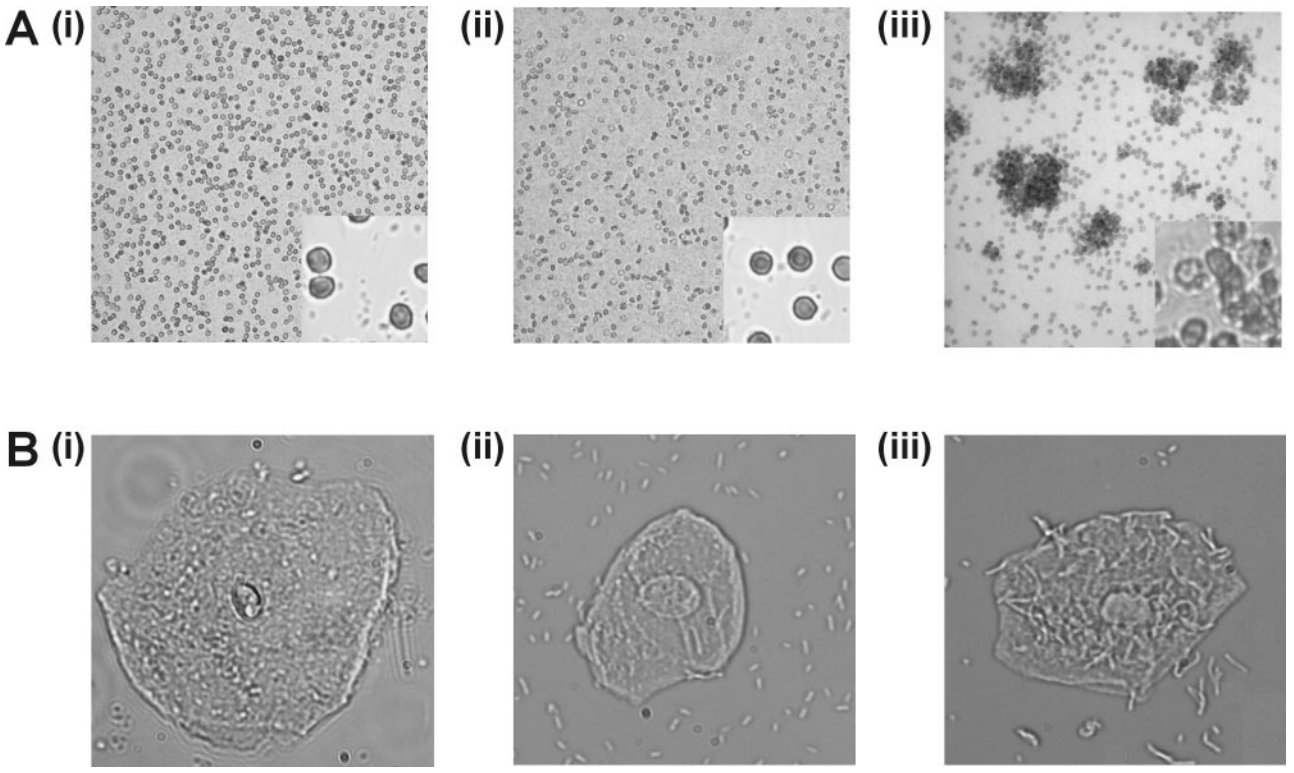

FIG. 3. Agglutination of human type A RBC (A) and binding to human uroepithelial cells (B) by (i) *E. coli* 83972, (ii) *E. coli* 83972 (pDD3; contains all *pap* genes from UPEC strain J96 except *papG*), and (iii) *E. coli* 83972 (pDD4; *papG* gene from J96). Functional activity was observed only in the presence of the plasmid containing the *papG* gene from *E. coli* J96. Shown are the binding phenotypes under low (10×) (A) and high (63×) (B) magnifications.

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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83972: MKKWFPAFLFLSLSGCNDALAI-QSTMFYSFNDNIYRPRLSVKVTDVIQIIVDINSASST
536  : .....AN...I.....H.Q.....IV.F..... ...

83972: ATLSYVDCNGFTWSHGIYWSEYFAWLVVPKRVSYNGYDIYLELQSRGSFSLDAEDND NYY
536  : .....A.....T.L.....H.....N.......K.G.....  .  .

83972: LTKGFAWDEANTSGRTCFNIGEKRSLAWSFGGVTLNARFPVDLPKGDYTFPVKFLRG IQR
536  : .........V.S...V..D.....L.....  ...

83972: NNYDYIGGRYKIPSSLMKTFPFNGTLNFSIKNTGGCRPSAQSLEINHGDLSINSANN HYA
536  : ..... ...

83972: AQTLSVSCDVPTNIRFFLLSNTAPAYSHGQQFSVGLGHGWDSIVSVNGVDKGETTMR WYR
536  : .....T.....I....T..... ...

83972: AGTQNLTIGSRLYGESSKIQPGVLSGSATLLMILP
536  : .....
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FIG. 4. Alignment of the amino acid sequence deduced from the nucleotide sequence of the *E. coli* 83972 *papG* gene with the closely related sequence of the uropathogenic *E. coli* 536 *prfG* gene. Residues identical to those of the *E. coli* 83972 *papG* gene are indicated by dots; gaps introduced into the alignment are indicated by dashed lines. The residues conserved between PapG I, II, and III alleles are shaded.

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