CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 2001, p. 637–640 Vol. 8, No. 3
1071-412X/01/$04.00+0 DOI: 10.1128/CDLI.8.3.637–640.2001

Prevalence of Known P-Fimbrial G Alleles in *Escherichia coli* and Identification of a New Adhesin Class

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Received 2 October 2000/Returned for modification 28 December 2000/Accepted 14 February 2001

Screening a large *Escherichia coli* collection for P-fimbrial adhesin classes identified 20 unclassifiable strains. Cloning and sequencing of *papG* from an unclassifiable strain identified another G allele. The novel adhesin gene has 65% identity to the class I adhesin gene, 44% identity to the class II adhesin gene, and 43% identity to the class III adhesin gene.

P-fimbrial adhesins in *Escherichia coli* enable the colonization of host tissues. By mediating attachment to P-blood group antigens on uroepithelial cells (6, 12, 16), P fimbriae play a critical role in the development of urinary tract infections (UTIs). P fimbria production is regulated by a chromosomal *pap* operon, containing 11 genes (4). The P-fimbrial-tip adhesin, which is encoded by *papG*, attaches directly to host cells (7, 9). The three adhesin classes (*papG*$_{J96}$ [class I], *papG*$_{AD/IA2}$ [class II], and *prsG*$_{J96}$ [class III]) were characterized based on their capacities for binding to specific Gal(α1-4)Gal-containing glycolipids (14). *prf* (pap-related fimbriae) is generalized nomenclature that includes all gene clusters encoding P fimbriae. The *prf* probe contains the most conserved genes and thus hybridizes to all of these gene clusters (1). Class I adhesins have 45% identity at the amino acid level to class II and 46% identity to class III, while class II adhesins have 56% identity to class III. Minor *papG* variants have been reported (GenBank accession numbers AAF61952 [J. R. Johnson, N. Kaster, T. T. O'Bryan, and A. L. Stell, unpublished data], AAF61956 [5], AAD13607 [3], and AAA59216 [10]) with homology to the three *papG* alleles, ranging in identity from 89 to 96%. We present the discovery of a new G allele of P fimbriae with less than 65% identity to known adhesin classes.

Three *E. coli* collections were studied: 313 isolates from college women aged 18 through 39 at the University of Michigan or University of Texas at Austin between 1992 and 1995 with a first-time UTI (first UTI), 51 isolates from a subset of these same women reporting a second UTI within 6 months of the first (second UTI), and 377 fecal and 74 periurethral isolates from healthy women presenting to the University of Michigan Student Health Service for gynecological exams between February and March 1996. All *E. coli* isolates were cultured and processed as previously described (1, 17).

A total of 815 *E. coli* strains were screened by dot blot hybridization for the presence of *prf*, a cluster of gene sequences specific to P-related fimbriae, as described previously (1). Sequence homology to *prf* was detected in 332 (41%) of the strains tested. The P-fimbrial adhesin class was also determined by screening those strains positive for *prf* with the three class-specific DNA probes using dot blot hybridization (Table 1). The adhesin class-specific probes (*papG*$_{J96}$ [class I], *papG*$_{AD}$ [class II], and *prsG*$_{J96}$ [class III]) were derived from published sequences (8, 13, 14) and isolated from control strain J96 or C1212 by PCR (1, 2). Strain J96 contains *papG*$_{J96}$ and *prsG*$_{J96}$, while control strain C1212 contains *papG*$_{AD/IA2}$. We confirmed dot blot results with PCR using unique primers for each adhesin class. Identical PCR conditions, except the annealing temperatures, were used for each adhesin class (30 cycles of 94°C for 60 s and 73°C for 40 s, with an annealing time of 35 s). Table 2 lists the annealing temperatures and PCR primers. Based on these results, 20 strains positive for *prf* and negative for all three adhesin classes were identified (Table 1), suggesting the presence of *papG* variants.

Pulsed-field gel electrophoresis analysis showed that all 20 strains differ by three or more bands (15) and therefore do not represent a clonal grouping (data not shown). Because *papF* is conserved among the three known adhesin classes (13), we

TABLE 1. P-fimbrial adhesin classes by strain source

Population	No. (%) of *prf*-positive strains	No. (%) of isolates carrying adhesin gene[a]			
		papG$_{J96}$ (class I)	*papG*$_{AD/IA2}$ (class II)	*prsG*$_{J96}$ (class III)	Unknown class
First UTI (n = 313)	153 (48.9)	5 (1.6)	85 (27.2)	62 (19.8)	8 (3.3)
Second UTI (n = 51)	24 (47.1)	0 (0)	10 (19.6)	10 (19.6)	1 (2.4)
Fecal (n = 377)	128 (34.0)	0 (0)	88 (23.3)	31 (8.2)	11 (4.1)
Periurethral (n = 73[b])	27 (37.0)	0 (0)	13 (17.8)	13 (17.8)	0 (0)

[a] The subclass totals exceed the *prf*-positive totals for the first-UTI and fecal populations because some of these strains have more than one adhesin class.
[b] Data missing for one periurethral strain.

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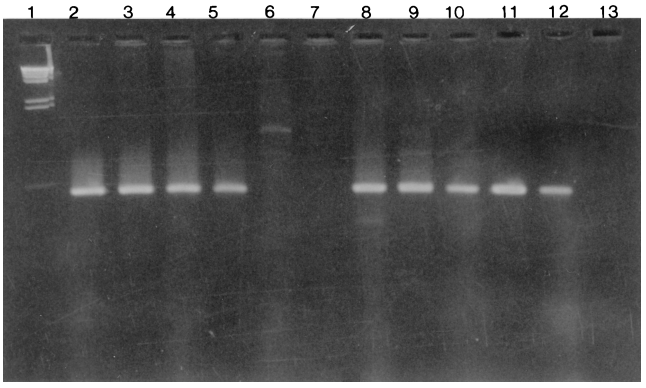

FIG. 1. PCR amplification of *papF*. Lane 1, DNA lambda digested with *Hin*dIII; lane 2, J96; lanes 3 to 12, unknown-adhesin strains BF31, BF56, BF1163, BF141, BF192, BF115, BF1160, BF166, and BF268; lane 13, negative control. PCR amplification of *papF* was detected using primers 5′-ATCGTTGCTTCTGACATCGG-3′ and 5′-GTCAA TAAGTAATCCCATA CTG-3′ (30 cycles of 94°C for 60 s, 56°C for 30 s, and 74°C for 30 s).

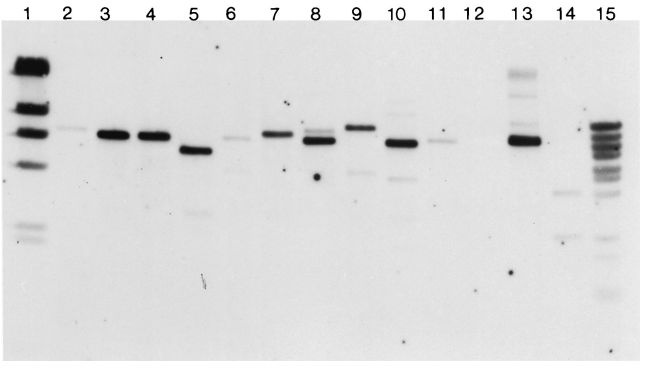

FIG. 2. Restriction fragment length polymorphisms of *E. coli* strains digested with *Bsa*B1 and probed with *papF* (502 bp). Lanes 1 and 15; γ DNA markers digested with *Hin*dIII and *Bst*EII; lanes 2, 3, 4, 5, 7, 8, 9, l0, and 12, unknown-adhesin fecal strains BF6, BF31, BF54, BF56, BF164, BF166, BF191, BF224, and BF370; lanes 6, 11, and 13, unknown-adhesin UTI strains BF115, BF268, and BF1009; lane 14, control strain J96.

assessed whether it could be amplified using PCR in the 20 strains (Fig. 1). A 502-bp fragment was amplified in control strains J96 and C1212 and in 11 of 20 (55%) strains with an unknown *papG* adhesin class.

In order to determine whether any of these strains contained novel P-fimbrial G alleles, we cloned and sequenced *papG* from two randomly selected strains (BF1163 and BF31). Southern blot hybridization using a 502-bp *papF* PCR probe labeled with digoxigenin (Genius System kit; Boehringer Mannheim, Indianapolis, Ind.) detected DNA fragments of 6.5 kb for fecal strain BF31 (Fig. 2), and of 4 and 2.3 kb for UTI strain BF1163 digested with *Bsa*B1 and *Psp*14061 (data not shown), respectively. Both the 6.5- and the 4-kb DNA fragments were purified following gel electrophoresis and cloned using the pZErO-1 vector (Invitrogen, San Diego, Calif.) with TOP10F′ (Invitrogen) as the recipient strain by methods described previously (17). Plasmid DNA was isolated using a plasmid preparation kit (Qiagen, Chatsworth, Calif.). Restriction enzyme digestion, nuclease treatment, and ligation were performed according to standard protocols (11).

The double-stranded DNA sequences of both clones were determined at the University of Michigan Biology Core Facility with an Applied Biosystems model 373A automated sequencer using primers T7 and SP6. Fecal strain BF31 contained a novel *papG* allele ($papG_{BF31}$), whereas UTI strain BF1163 contained a variant of *papG* with a deletion. $papG_{BF31}$ had amino acid sequence identities of 65% to $papG_{J96}$ (class I), 46% to $papG_{AD/IA2}$ (class II), and 45% to $prsG_{J96}$ (class III) (Fig. 3). We refer to $papG_{BF31}$ as the P-fimbrial class IV adhesin gene. BF1163 was most similar (70%) to $papG_{J96}$; however, the open reading frame was truncated at bp 290.

In order to estimate the prevalence of $papG_{BF31}$ in other *E. coli* strains, we screened a sample of strains ($n = 308$) positive for *prf* by dot blot hybridization using a 371-bp probe specific to $papG_{BF31}$. $papG_{BF31}$ occurred with similar frequency in each collection. The numbers of strains positive for $papG_{BF31}$ were as follows: 21 (15%) among the first UTI collection ($n = 144$), 36 (15%) among the second UTI collection ($n = 20$), 20 (17%) among the fecal collection ($n = 120$), and 3 (13%) among the periurethral collection ($n = 24$). $papG_{BF31}$ is positively associated with *aer* and *drb* and is negatively associated with $prsG_{J96}$, *hly, cnfl, ompT,* and *sfa* (Table 3). Among isolates positive for $papG_{BF31}$, $papG_{AD}$ was present in 55%.

PCR was performed on the remaining 19 *prf*-positive, class I-, II-, and III-negative strains using primers 5′-GACTATTC TGGTTATGATTC-3′ and 5′-CAATGAATTAAGGTTTAG-3′ (30 cycles of 95°C for 60 s, 46°C for 40 s and 73°C for 23 s), taken from a unique coding region of $papG_{BF31}$. A 371-bp fragment was amplified in 8 of the 19 (42%) strains, suggesting that other novel G allele variants may exist.

The novel class IV adhesin gene shows 45 to 65% similarity at the amino acid level to the three adhesin classes, thereby representing a unique adhesin class that is found equally among UTI and fecal *E. coli* strains. Thus, class IV adhesins are not exclusively associated with UTIs, although they could be associated with the pathogenesis of other important diseases.

The large *prf* probe used in this study hybridized to strains containing novel *papG* alleles as well as inactive *papG* variants

TABLE 2. Oligonucleotides used in PCR typing

PCR primer	Oligonucleotide	Strand	Size (bp)	Annealing temp (°C)
Conserved	5′-ACCACGGCCAGTATGAGCATG-3′	+		
Class I ($papG_{J96}$)	5′-CAGGATAGAAACATATACGGGCA-3′	−	449	58
Class II ($papG_{AD}$)	5′-AATCTGGCGTTCAGGGTAACAC-3′	−	551	62
Class III ($prsG_{J96}$)	5′-CCAAGTAACTCGGGAAATGAC-3′	−	637	61

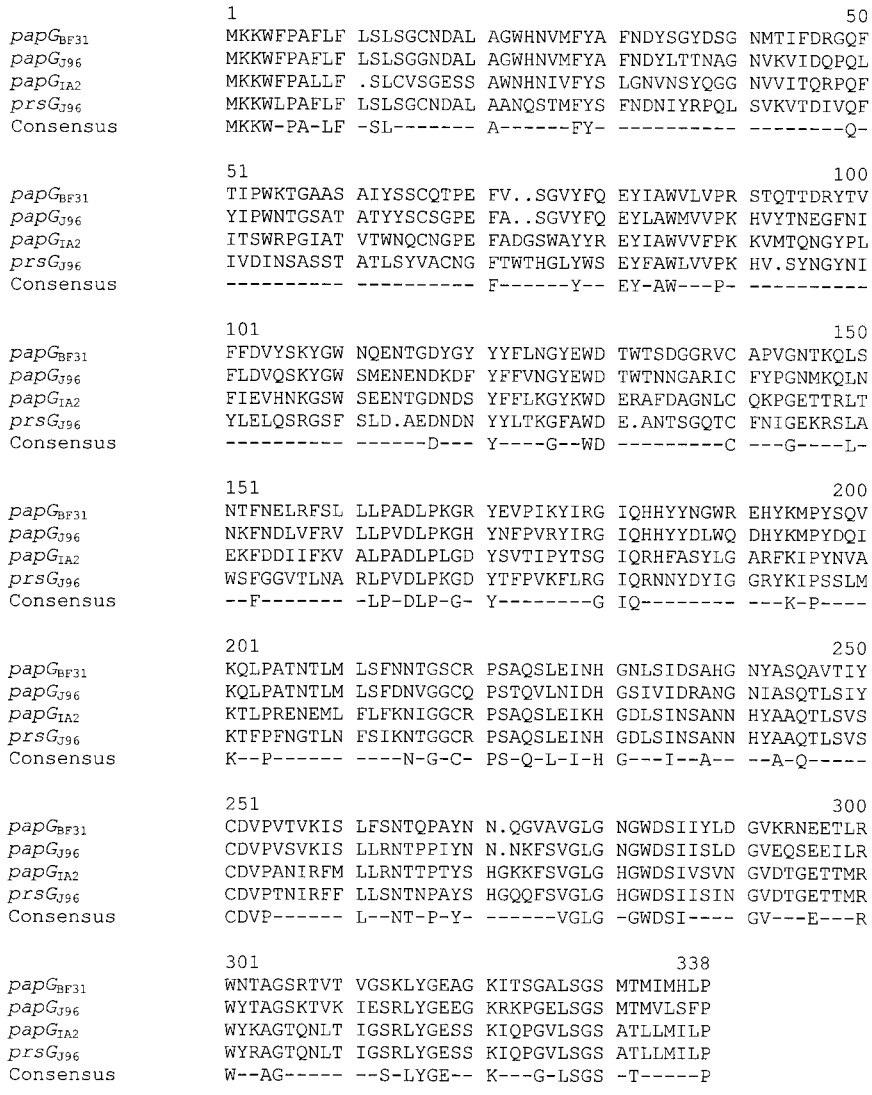

FIG. 3. Amino acid comparison of the new class IV adhesin (encoded by $papG_{BF31}$) with the three known adhesin classes. DNAStar (Madison, Wis.) and Genetics Computer Group (Madison, Wis.) software was used for DNA and amino acid analyses.

or variants with deletions. Because a PCR fragment specific to the class IV gene was not amplified in 11 of the strains without class I, II, or III adhesins, it is possible that other novel molecular variants of *papG* exist. Future work should include hemagglutination assays to determine whether $papG_{BF31}$ is functional and to identify other novel *papG* variants and assess their role in UTIs or other diseases.

Nucleotide sequence accession number. The GenBank accession number for the $papG_{BF31}$ nucleotide sequence is AF304159.

TABLE 3. Prevalence of virulence factor genes among the entire collection and among the subset positive for $papG_{BF31}$

Sample	No. (%) positive for selected virulence factor gene										
	$papG_{AD}$	$papG_{J96}$	$prsG_{J96}$[b]	*aer*[b]	*kpsMT*	*hly*[b]	*cnf1*[b]	*ompT*[b]	*drb*[b]	*sfa*[b]	*fim*
Total collection (n = 308)[a]	183 (59)	5 (2)	105 (34)	142 (46)	275 (89)	152 (49)	105 (34)	282 (92)	19 (6)	112 (36)	308 (100)
$papG_{BF31}$ (n = 47)	26 (55)	2 (4)	1 (2)	35 (74)	40 (85)	10 (21)	2 (4)	30 (64)	11 (22)	4 (9)	47 (100)

[a] The virulence factor genes encode the following: aerobactin (*aer*), group II capsules (*kpsMT*), α-hemolysin (*hly*), cytotoxic necrotizing factor 1 (*cnf1*), outer membrane protease T (*ompT*), afimbrial adhesins I to IV and F1845 pili (*drb*), S fimbriae (*sfa*), and type 1 fimbriae (*fim*). The data are the numbers (percentages) of isolates containing each gene (e.g., among those strains positive for $papG_{BF31}$, 55% were also positive for $papG_{AD}$).

[b] The proportion positive for a selected gene among isolates with $papG_{BF31}$ is significantly ($P < 0.0001$) different from the proportion in the total collection.

We thank Charlotte Williams, Ronald Mulder, and the laboratory and gynecologic clinic staffs at the University of Michigan Student Health Service as well as Scott Spear, Shirley Arldt, Nell Curtis, Barbara Locke, and the staff at the University of Texas at Austin Student Health Service for collecting bacterial specimens, and we thank Karin Palin for her intellectual contributions.

This work was supported by Public Health Service grants DK-35368 and DK-55496 from the National Institutes of Health.

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