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Molecular Cloning of *Proteus mirabilis* Uroepithelial Cell Adherence (*uca*) Genes

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Proteus mirabilis **bacteria are a common cause of hospital-acquired urinary tract infection. In a previous study, we described a *P. mirabilis* fimbrial protein, UCA, that adhered to human uroepithelial cells. Genes sufficient for expression of UCA adherence were cloned into *Escherichia coli* K-12. *E. coli* bacteria that contained the *uca* recombinant plasmid adhered to human uroepithelial cells. In addition, the *ucaA* gene encoding the structural component of UCA pili was subcloned, and its DNA sequence was determined. Amino acid sequence homology (30 to 50%) was found between mature UcaA protein and pilins from pathogenic bacteria representing several genera, including *E. coli* F17, G, and type 1C pilins, *Haemophilus* M43 pilin, and a *Bordetella* pilin.**

Urinary tract infections are the most common nosocomial disease. The microbiology of nosocomial urinary tract infections, especially in elderly and handicapped persons, is different from that of acute community-acquired disease. Unlike acute urinary tract infections, wherein a single bacterial species, *Escherichia coli*, accounts for over 90% of infections, nosocomial urinary tract infections may be caused by a variety of bacterial genera, including several members of the family *Proteeae*, in particular, *Proteus mirabilis* (17). Depending upon the method of urinary tract management, the incidence of *P. mirabilis* in positive urine cultures of elderly patients or individuals with spinal cord injury ranges from 10 to 80% (11). Infections with *P. mirabilis* are especially significant in that these bacteria produce a more severe disease than *E. coli* as a result of the production of urease, which promotes tissue damage and struvite stone formation (9, 15).

In addition to urease production, several bacterial properties are associated with urovirulent strains of *P. mirabilis*, including motility, invasiveness in tissue culture, hemolytic activity, and certain protocine types (24, 27–30, 39). The adherence of *P. mirabilis* to urinary tract tissue is another property thought to contribute to virulence (6, 35–38). In other pathogenic bacteria, tissue adherence is mediated by bacterial surface structures, including capsules, glycocalyxes, and pili (5). For example, P pili mediate attachment of uropathogenic *E. coli* to human urinary tract epithelium (14). However, P pili are not found among other genera of the family *Enterobacteriaceae* (13). The mechanism by which *P. mirabilis* adheres to uroepithelium is unclear. *P. mirabilis* may express a variety of adherence organelles which have been characterized by their ability to agglutinate various erythrocyte types (MRK and MRP pili, for example) or to adhere to uroepithelium (UCA fimbriae) (26, 44). In general, little correlation has been found in *P. mirabilis* between hemagglutination potential and adherence to uroepithelium (1, 22), although Sareneva et al. (34) have reported an association between MRP hemagglutination and uroepithelial cell adherence in a *P. mirabilis* clinical isolate. This is in contrast to uropathogenic *E. coli*, where the principle adhesin, P pili, is also a hemagglutinin.

In a previous study, we reported isolation from a uropathogenic *P. mirabilis* of a protein (UCA) that adhered to desquamated uroepithelial cells; it was shown to be organized as pili on the surface of the bacteria (44). In the current study, we report the molecular cloning of genes associated with *P. mirabilis* UCA adherence.

Molecular cloning of *P. mirabilis* UCA genes. In 1986, Wray et al. (44) reported the amino-terminal acid sequence of the UCA structural protein. This sequence was used to search the Swissprot protein sequence databank for a similar sequence. Significant homology was found between the sequence of UCA and the deduced amino acid sequence of the *E. coli* F17 pilin structural protein (19). Partial amino acid sequences obtained from internal tryptic digest fragments of UCA were also similar to those of F17 fimbriae. With the DNA sequence of the F17 structural gene as a guide, and taking into account observed differences in UCA amino acid sequence, two converging oligonucleotide primers were prepared. The sequences of primers used for amplification of UCA were CTCATAAGC GATGGTGTAATGAACTGTAGC and TATGACGGTAC AATTACTTTTACTGGAAA. These primers were used together with *P. mirabilis* HU1069 DNA in a PCR to amplify a portion of the UCA structural gene, designated *ucaA*. *P. mirabilis* HU1069 is a urinary tract isolate obtained from Ben Taub County Hospital, Houston, Tex. The resulting PCR product was then used as a hybridization probe to identify clones containing *ucaA* in a cosmid library prepared from *P. mirabilis* HU1069. Methods used for cosmid cloning have been described previously (12). Cosmids were packaged with Gigapack II XL packaging extracts (Stratagene). Standard procedures were used for plasmid purification, restriction endonuclease digestions, DNA ligations, and agarose gel electrophoresis (32). Nineteen of 4,800 cosmid-containing colonies screened by colony blot hybridization reacted with the probe. One of these cosmids, designated pRHU1333, was selected for further study.

An 11-kb *Hin*dIII fragment containing the UCA structural gene was subcloned from pRHU1333 into a new plasmid vector, pBluescript II BC KS$^+$, to form pRHU1456. A restriction endonuclease map depicting pRHU1456 and various subclones derived from pRHU1456 is shown in Fig. 1.

Adherence to desquamated urinary tract epithelium. The capacity of *E. coli* K-12 bacteria containing the recombinant

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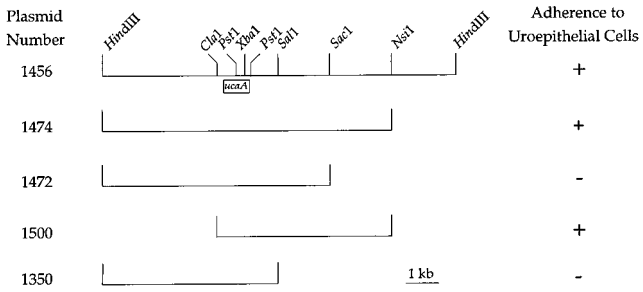

FIG. 1. Restriction endonuclease site map of pRHU1456 and derivative subclones. Plasmid vectors (not shown) are: pBluescript II BC KS for pRHU1456; pBluescript II SK for pRHU1472 and pRHU1474; and pACYC184 for pRHU1350.

plasmid pRHU1456 and various subclones of pRHU1486 to adhere to desquamated uroepithelium was compared with that of the donor *P. mirabilis* HU1069 as described previously (44). The results in Table 1 show that *E. coli* transformed with pRHU1456 or subclones of pRHU1456 that contain the 5-kb *Hin*dIII-*Nsi*I fragment adhere to uroepithelial cells. For both *P. mirabilis* HU1069 and the *E. coli* recombinant, there was no morphological difference between epithelial cells that contained adherent bacteria (i.e., cells expressing UCA receptor) and those that did not bind bacteria.

Hemagglutination phenotype. *E. coli* XL1-Blue(pRHU1456) and *P. mirabilis* HU1069 were grown under conditions optimal for expression of uroepithelial cell adherence and tested for the ability to agglutinate untreated ox, fowl, or human erythrocytes or tanned human or ox erythrocytes. No hemagglutination was observed with either *P. mirabilis* HU1069 or the recombinant *E. coli* strain. Similar results have been reported previously for *P. mirabilis* HU1069 (23, 44).

In vitro translation. Radiolabeled proteins expressed by plasmid pRHU1500 and the vector plasmid pBluescript II SK were produced by in vitro translation in the presence of ^{35}S-methionine. In vitro translation of recombinant plasmids was done with a procaryotic in vitro transcription-translation kit (Amersham Life Sciences) as described in the manufacturer's instructions. The products were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by autoradiography (18). The results are shown in Fig. 2. In vitro translation of pRHU1500 resulted in at least seven peptide bands in addition to those encoded by the vector.

DNA sequence analysis of *ucaA*. Southern blot hybridization, with *ucaA* PCR product as a probe, was used to localize *ucaA* to a 6.5-kb *Sal*I fragment of cosmid pRHU1333. This fragment was subcloned into pACYC184 to produce pRHU1350 (Fig. 1), and the DNA sequence of the *ucaA* structural gene was determined. The DNA sequence was obtained from a double-stranded template with a Sequenase 2.0 DNA sequencing kit (U.S. Biochemicals) as described in the manufacturer's instructions. Both strands of the template were sequenced completely. The nucleotide sequence of *ucaA* and the amino acid sequence of the deduced protein are shown in Fig. 3. Sequence analysis revealed the presence of an open reading frame encoding 180 amino acids starting with a methionine at position 144. The N-terminal sequence reported previously for mature UCA protein was found to match the deduced amino sequence beginning at position 210. The ATG codon at position 144 was preceded by a potential ribosome binding site (AGGAA) (position 129). The deduced amino acid sequence between positions 144 and 210 has properties typical of a signal peptide. The remaining amino acid sequence predicts a mature protein with a molecular weight of 16,700. The results of a Western blot (immunoblot) analysis, depicted in Fig. 4, confirm that the recombinant strain HU1350 produces a new antigen reactive with anti-UCA sera.

TABLE 1. Adherence of *P. mirabilis* HU1069 and *E. coli* derivatives to human uroepithelial cells

Bacterial strain	Fraction of uroepithelial cells with adherent bacteria (%)[a]
HU1069	60/1,274 (4.9)
HU1350	0/200 (0)
HU1456	19/884 (2.2)
HU1472	0/830 (0)
HU1474	10/714 (1.4)
HU1500	11/342 (3.5)

[a] Uroepithelial cells containing adherent bacteria, for both *P. mirabilis* and adherent *E. coli* clones, had greater than 30 bacteria attached per uroepithelial cell, covering the entire surface of the cell.

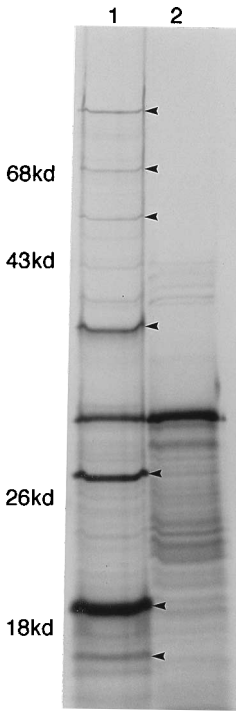

FIG. 2. Autoradiograph of SDS-PAGE showing proteins translated in vitro from pRHU1500. Lanes: 1, pRHU1500; 2, pBluescript II SK. Bands unique to pRHU1500 are indicated by arrowheads.

The *ucaA* open reading frame was terminated by a stop codon at position 684. A possible transcriptional terminator is suggested by the inverted repeat located at positions 713 to 732. A second open reading frame was found; it began with an ATG at position 752 and was preceded by a potential ribosome binding site at position 743.

Codon usage frequency of *ucaA*. Table 2 shows codon use frequency analysis for the *ucaA* gene in comparison with that of seven other *P. mirabilis* genes, including *ureA*, *-B*, *-C*, *-E*, and *-F* (16), *pmfA* (3), and *mrpA* (4). Also included in Table 2 are codon frequencies for *H. influenzae* M43 pili (8), *E. coli* P pili (2) and typical *E. coli* genes (10).

Numerous studies with both diarrheagenic and uropathogenic *E. coli* have demonstrated the importance of adherence

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        10                  30                  50
ttttttattaatttttaatatttgaaaatcaataaataactttgttgtttttaaatttta

        70                  90                  110
ttcattattcaaatagtaatttaatttttttgttaaaaaaataaaaataacctaacttttta

       130                 150                 170
tttaaaataggaatcataaatatatgaaaagaaaagttatagcactagctactattcttt
                         MetLysArgLysValIleAlaLeuAlaThrIleLeuS

       190                 210                 230
ctgctgcatttgctggctcatctatggcgtatgacggaacaattacatttacaggtaaag
erAlaAlaPheAlaGlySerSerMetAlaTyrAspGlyThrIleThrPheThrGlyLysV

       250                 270                 290
ttgttgcgcaaacctgctctgtcaatacaaatgataagaatttagcggtaacattaccta
alValAlaGlnThrCysSerValAsnThrAsnAspLysAsnLeuAlaValThrLeuProT

       310                 330                 350
cagtatccaccactacattaaatgaaaatgcggctactgcaggtcttactccatttacta
hrValSerThrThrThrLeuAsnGluAsnAlaAlaThrAlaGlyLeuThrProPheThrI

       370                 390                 410
ttcatttaactggttgcgctgttggtatggatggtgcacaaagtgtcaaaacatattttg
leHisLeuThrGlyCysAlaValGlyMetAspGlyAlaGlnSerValLysThrTyrPheG

       430                 450                 470
aaccttcaagtgacattgatgtaaccacacacaacttaaaaaatactgcacaaactaaag
luProSerSerAspIleAspValThrThrHisAsnLeuLysAsnThrAlaGlnThrLysA

       490                 510                 530
ctgataatgttcaagttcaattacttaactcagatgcagcaacaacaatccagttaggta
laAspAsnValGlnValGlnLeuLeuAsnSerAspAlaAlaThrThrIleGlnLeuGlyT

       550                 570                 590
ctgattctgcaacacaagatgtccatccagtacaaatcgacaatgctaatgtaaacctcc
hrAspSerAlaThrGlnAspValHisProValGlnIleAspAsnAlaAsnValAsnLeuP

       610                 630                 650
catattttgctcaatattatgcaaccggacaatctaccgctggggatgtaaaagcaaccg
roTyrPheAlaGlnTyrTyrAlaThrGlyGlnSerThrAlaGlyAspValLysAlaThrV

       670                 690                 710
ttcattacaccattgcctatgagtaaggtttattggattgcttttatttacggggcaga
alHisTyrThrIleAlaTyrGluEnd

       730                 750                 770
aaaaactgccccatttccttctagaggtattatgttaaagttcattcttattttcttgac
                         MetLeuLysPheIleLeuIlePheLeuTh

       790                 810                 830
tctgtttgtatccacagtggctgaagcagtgtgttattatggggaccgcgttgtttatcc
rLeuPheValSerThrValAlaGluAlaValCysTyrTyrGlyAspArgValValTyrPr

       850                 870                 890
cgcaacgcaaaaaagcattaatgttcaattaaataataacgatgaatccccgcatt
oAlaThrGlnLysSerIleAsnValGlnLeuAsnAsnAsnAspGluSerProHis
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FIG. 3. Nucleotide sequence of the *ucaA* gene. The deduced amino acid sequence is shown below the nucleotide sequence. Putative regulatory sequences are underlined.

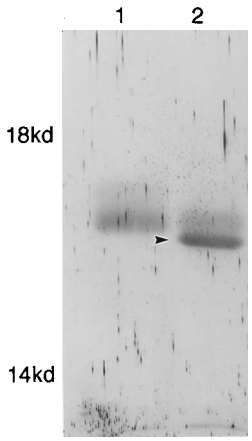

FIG. 4. Western blot analysis of UCA pilin produced by *E. coli* XL1-Blue(pRHU1350). Whole-cell bacterial proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes (32). UCA antigens were detected with anti-UCA polyclonal antisera (3). The position of the *ucaA* gene product produced by *P. mirabilis* HU1069 is indicated by an arrowhead. Lanes: 1, *E. coli* XL1-Blue(pACYC184); 2, *E. coli* XL1-Blue(pRHU1350).

to host tissue as a bacterial virulence factor. We postulated that specific adherence of *P. mirabilis* to uroepithelial tissue would also be an important contributor to urovirulence. In initial studies, we identified a *P. mirabilis* surface protein, called UCA, that adhered to desquamated uroepithelial cells (44).

In the current study, the genes encoding functional UCA adherence have been cloned, and the DNA sequence of the *ucaA* structural gene has been determined. *E. coli* K-12 containing the cloned *P. mirabilis* genes acquired the uroepithelial cell-adherent phenotype of the *P. mirabilis* DNA donor. The recombinant clone encoded synthesis of at least seven peptides in vitro. While additional characterization of each cloned gene is anticipated and will be required before gene-protein or protein-function assignments can be made, the 18.5-kDa in vitro peptide may be the unprocessed product of the *ucaA* gene on the basis of its size, its presence in in vitro translations of both pRHU1500 and pRHU1350, and its absence in in vitro translations of *Pst*I-digested pRHU1350 (data not shown).

The UcaA protein deduced from the DNA sequence possesses physical characteristics common to many pilins: a signal sequence at the N terminus, a cysteine loop in the amino half of the protein, and a penultimate tyrosine at the C terminus. In a previous study, UcaA was shown to be organized as pili on the surface of *P. mirabilis*. In spite of this, our initial electron micrographs have failed to demonstrate the presence of pili on our recombinant *E. coli* strains. This may be because accessory genes are absent as a result of their location at a distant unlinked site on the *P. mirabilis* chromosome or as a result of other problems associated with pilus formation in our *E. coli* host. Further experiments in which growth conditions will be varied are planned to induce pilus production. Thus, at present, we cannot conclude whether UCA adherence in the recombinant strains may or may not be due to polymerized pilin structures. The signal peptide has several typical features, including a hydrophobic amino terminus containing 3 basic amino acids, a central hydrophobic core consisting of 10 amino acids, and an alanine residue adjacent to the cleavage site (31). In a previous report, the mature protein was shown to start at the tyrosine residue at position 210 (44). The *uca* gene is preceded by a putative ribosome binding site and a −10 promoter sequence at position 52 (TAAATT). A −35 sequence (TTGAAA) similar to the consensus TTGACA sequence for sigma 70 promoters was found at position 22 (42). A potential transcriptional terminator was also found; however, results of the in vitro translation experiments suggest that some readthrough may occur.

The codon usage frequency shown for *ucaA* is similar to that found for other *P. mirabilis* genes, with a preference for uridine and adenine in the third position. In addition, the base composition of *ucaA* (34% G+C) is characteristic of *P. mirabilis* (39% G+C) (43), suggesting that the origin of *uca* is within this genus.

The DNA sequence and deduced amino acid sequence of UCA were found to be similar to those of an *E. coli* fimbrial antigen, called F17, associated with bovine diarrhea (19). The DNA sequence of UCA was 59% homologous to that of the F17A gene, which encodes the structural protein for F17 fimbriae. Similarity between the deduced amino acid sequence of each protein is also apparent; there was 58% identity between the primary structures of UCA and F17A proteins over an alignment range of 170 amino acids (Fig. 5). Codon usage frequency and base composition of the F17A gene more closely resembled those of *ucaA* and other *P. mirabilis* genes than those of other *E. coli* genes or the *E. coli papA* pilin gene

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TABLE 2. Comparison of codon usage of the *P. mirabilis* UCA pilin gene with codon usage of other pilin and nonpilin genes

Amino acid	Codon	Frequency of codon use (%)					
		uca	Other *P. mirabilis* genes	F17a pilin	M43 pilin	Pap pilin	Other *E. coli* genes
Gly (F)	GGG	10	12	15	8	12	10
	GGA	20	12	31	23	17	6
	GGU	60	48	46	62	54	44
	GGC	10	28	8	8	17	40
Glu (E)	GAG	0	36	0	20	33	27
	GAA	100	64	100	80	66	73
Asp (D)	GAU	73	84	86	86	91	49
	GAC	27	16	14	14	9	51
Val (V)	GUG	27	24	35	24	14	26
	GUA	27	20	6	41	23	21
	GUU	40	43	53	35	59	38
	GUC	7	13	6	0	5	14
Ala (A)	GCG	0	14	12	18	9	28
	GCA	38	35	28	53	18	23
	GCU	44	38	56	24	45	26
	GCC	19	13	4	6	27	23
Arg (R)	AGG	0	4	0	0	0	3
	AGA	100	8	0	0	0	5
	CGG	0	4	0	0	0	6
	CGA	0	15	0	0	0	4
	CGU	0	44	100	0	0	47
	CGC	0	26	0	0	0	35
Ser (S)	AGU	18	25	7	25	0	12
	AGC	0	11	13	17	14	20
	UCG	0	8	7	0	14	13
	UCA	27	24	20	25	29	8
	UCU	45	31	53	25	29	23
	UCC	9	1	0	8	14	24
Lys (K)	AAG	12	10	25	11	31	26
	AAA	88	90	75	89	69	74
Asn (N)	AAU	75	77	87	92	67	31
	AAC	25	23	13	8	33	69
Ile (I)	AUA	12	10	10	0	11	5
	AUU	62	62	70	87	78	35
	AUC	25	28	20	13	11	60
Thr (T)	ACG	0	13	22	25	8	19
	ACA	43	25	35	25	15	8
	ACU	32	32	35	39	69	27
	ACC	25	30	9	11	8	46
Cys (C)	UGU	0	77	50	100	50	43
	UGC	100	23	50	0	50	57
Tyr (Y)	UAU	86	80	50	50	100	44
	UAC	14	20	50	50	0	56
Leu (L)	UUG	0	11	21	13	11	8
	UUA	58	54	21	44	11	8
	CUG	0	11	21	6	44	62
	CUA	8	5	7	6	11	2
	CUU	25	14	29	25	22	10
	CUC	8	5	0	6	0	10
Phe (F)	UUU	100	72	50	78	56	47
	UUC	0	28	50	22	44	53
Gln (Q)	CAG	10	21	80	11	100	72
	CAA	90	79	20	89	0	28
His (H)	CAU	75	68	0	60	100	46
	CAC	25	32	0	40	0	54
Pro (P)	CCG	0	12	0	0	20	61
	CCA	60	41	25	80	80	16
	CCU	40	29	75	20	0	12
	CCC	0	18	0	0	0	11

(Table 2), suggesting that *ucaA* and F17A may share a common ancestor. Like UCA, F17 fimbriae are tissue adherent, but they are not hemagglutinins. Considerable homology also exists between UCA and the amino-terminal end of the G hemagglutinin structural gene identified in a pyelonephritogenic *E. coli*; 15 of 19 amino acids were identical (33). Unfortunately, the amount of sequence information available for G fimbriae is limited.

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YDGTITFTGK VVAQTCSV-- -NTNDKNLAV TLPTVSTTTL NENAATAGLT      UCA
***K**N*** *D******-- -T*ES***T* K*****ANS* ASSGKVV***      F17
TS*KV**F** **EN**K*-- -K*EH***S* V*ND*GKNS* STKVN**MP*      M43
D***VI**T ITDT**VIED PSGPNHTKV* Q**KI*KNA* KA*GDQ**R*      BFMST2

PFTIHLTGC- AVGMDGAQSV KT-----YFE PSSDIDVTTH -NLKNTAQTK      UCA
****L*E**N TPAVT***N* NA-----*** *NANT*Y**G -I*T***SSG      F17
****T*QN*- DPTTANGTAN *ANKVGL**Y SWKNV*KENN FT***EQT*A      M43
**I*K*KD*- PSSL--GNG* *A-----*** *GPTT*YS*G -D*RAYKMVY      BFMST2

--ADNVQVQL LNSDAATTIQ LGTDSATQDV HPVQID-NA- NVNLPYFAQY      UCA
--*S***I** **A*GVKA*K **QAA*A*S* DT*A*N-D*- **T*R*N***      F17
DY*T**NI** MESNGTKA*S VVGKETEDFM *TNNNG-V*L *QTH*NN*HI      M43
--*T*P*T** S*IT***EA* -*VQVRISNL NDSK*TMG*- *EATQQA*GF      BFMST2

YATGQSTAGD VKATVHYTIA YE                                    UCA
****VA**** *TS**N**** *Q                                    F17
SGST*L*T*T NELPL*FIAQ *YATNKATAG KVQSSVDFQI AYE             M43
DPEV*TGGTS RTV*MR*LAS *VKKNGDVEA SAITTYVGFS VVYP            BFMST2
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FIG. 5. Comparison of the amino acid sequence of UCA protein with those of pilin proteins from other bacteria. UCA, *P. mirabilis* UCA; F17, *E. coli* F17; M43, *H. influenzae* type 6 M43; BFMST2, *B. pertussis* serotype 2 pilin. Asterisks indicate exact amino acid matches. Underlined amino acids represent functionally conserved changes as defined by Lipman and Pearson (20). Five additional amino acids (DINTE) at the amino end of M43 pilin are not shown.

Significant amino acid homology in the range of 30% was found between UCA pilin and pilin proteins from other genera, including *Haemophilus* (34%) (8, 41) and *Bordetella* (32%) (21), both associated with respiratory disease, *Klebsielliae* (31%) (7), and *E. coli* type Ic (28%) (40) (Fig. 5). All but *E. coli* type 1c pili are also hemagglutinins. However, for these bacteria, similarities in amino acid sequences were not reflected in DNA sequence homology. These results suggest functional similarity but less genetic relatedness between *ucaA* and this group of pilins. Only limited amino acid homology was found between UCA and *P. mirabilis* PMF (19%) and MRP (19%), *E. coli* P (15%), and *Serratia* type Ia (24%) (25) pilins.

These results suggest that UCA and F17 pilin subunits may represent the first family of adherence organelles with members represented in more than one enterobacterial genus. In addition, adhesins in this genetic family are found among bacteria associated with a variety of disease states, including respiratory, urinary, and enteric infections.

Several previous studies have shown little correlation between common *P. mirabilis* hemagglutinins and uroepithelial cell adherence. Our results are consistent with these observations. *P. mirabilis* HU1069 and *E. coli* HU1500 grown under conditions that promoted adherence to uroepithelial cells did not exhibit MRK- or MRP-type hemagglutination or agglutinate human erythrocytes. It remains possible that UCA^+ bacteria will agglutinate some other erythrocyte species, although all of the urinary tract infection *P. mirabilis* hemagglutinins reported by Adegbola et al. and Old and Adegbola (1, 26) agglutinated at least one of the erythrocyte types tested here. In contrast, Sareneva et al. reported that MRP hemagglutinating pili associated with a *P. mirabilis* clinical isolate mediated uroepithelial cell adherence (34). They also demonstrated adherence of purified MRP pili to human kidney substructures.

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The apparent molecular weight for the MRP pili used in their study was 21,000, clearly different from the apparent molecular weight of UCA pili (i.e., 17,500) (44). These results suggest that for uropathogenic *P. mirabilis*, more than one class of adhesin may promote bacterial attachment to uroepithelium. The virulence of *P. mirabilis* site-directed mutants defective in each of these adhesins must be determined with appropriate animal model systems before the contribution to virulence of each adhesin can be assigned.

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