Nucleotide sequences of two fimbrial major subunit genes, \textit{pmpA} and \textit{ucaA}, from canine-uropathogenic \textit{Proteus mirabilis} strains

Isaïc G. W. Bijlsma, Linda van Dijk, Johannes G. Kusters and Wim Gaastra

Proteus mirabilis strains were isolated from dogs with urinary tract infection (UTI) and fimbriae were prepared from two strains. The N-terminal amino acid sequences of the major fimbrial subunits were determined and both sequences appeared identical to the N-terminal amino acid sequence of a urinary cell adhesin (UCA) (Wray, S. K., Hull, S. I., Cook, R. G., Barrish, J. & Hull, R. A., 1986, \textit{Infect Immun} 54, 43-49). The genes of two different major fimbrial subunits were cloned using oligonucleotide probes that were designed on the basis of the N-terminal UCA sequence. Nucleotide sequencing revealed the complete \textit{ucaA} gene of 540 bp (from strain IVB247) encoding a polypeptide of 180 amino acids, including a 22 amino acid signal sequence peptide, and the \textit{pmpA} (\textit{P. mirabilis} P-like pili) gene of 549 bp (from strain IVB219) encoding a polypeptide of 183 amino acids, including a 23 amino acid signal sequence. Hybridization experiments gave clear indications of the presence of both kinds of fimbriae in many UTI-related canine \textit{P. mirabilis} isolates. However, the presence of these fimbriae could not be demonstrated in \textit{P. vulgaris} or other \textit{Proteus}-related species. Database analysis of amino acid sequences of major subunit proteins revealed that the UcaA protein shares about 56\% amino acid identity with the F17A and F111A major fimbrial subunits from bovine enterotoxigenic \textit{Escherichia coli}. In turn, the PmpA protein more closely resembled the pyelonephritis-associated pili (Pap)-like major subunit protein from UTI-related \textit{E. coli}. The evolutionary relationship of UcaA, PmpA and various other fimbrial subunit proteins is presented in a phylogenetic tree.

**Keywords**: Proteus mirabilis, fimbriae, major subunit gene, urinary tract infection

**INTRODUCTION**

\textit{Proteus} spp. can cause many kinds of extra-intestinal infections, such as otitis media and externa, meningitis, sepsis, and respiratory and urinary tract infections. The most frequent site of infection is the urinary tract and the majority of these infections is caused by \textit{Proteus mirabilis}, whereas \textit{Proteus vulgaris} is of minor importance (Adler et al., 1971). \textit{P. mirabilis} is not only an important cause of urinary tract infection (UTI) in humans but also in dogs. In both hosts, the majority of UTI is caused by \textit{Escherichia coli} and about 10\% by \textit{P. mirabilis} (Gaastra et al., 1995). To exert their pathogenicity, \textit{P. mirabilis} strains are equipped with several virulence factors, including the production of urease (Johnson et al., 1993; Jones et al., 1990), IgA proteases (Loones et al., 1992), haemolysin (Mobley et al., 1991), flagella (Allison et al., 1992) and fimbriae (Ahrens et al., 1993; Bahraei et al., 1991; Garcia et al., 1988b; Sareneva et al., 1990; Wray et al., 1986). In most bacterial infections, adherence is accepted as an indispensable virulence factor, in this case to protect the \textit{Proteus} bacteria from being washed out of the urinary tract by the urine flow.

Adherence in UTI by means of fimbriae has been extensively studied in both human and canine uro-
Table 1. Results of Southern blot analysis of PstI–HindIII-digested chromosomal DNA from a number of Proteus strains and Proteus-related species

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>IVB strain no.*</th>
<th>Source</th>
<th>Size of hybridizing fragments (kb)</th>
<th>UCA-band(s)†</th>
<th>Pmp-band‡ (Pap-like)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Proteus mirabilis</td>
<td>80</td>
<td>Dog urine</td>
<td>1 and 2.8</td>
<td>UCA-band(s)†</td>
<td>Pmp-band‡ (Pap-like)</td>
</tr>
<tr>
<td></td>
<td>219</td>
<td>Dog urine</td>
<td>5</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>225</td>
<td>Dog urine</td>
<td>2.8</td>
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</tr>
<tr>
<td></td>
<td>227</td>
<td>Dog urine</td>
<td>2 and 2.8</td>
<td></td>
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<tr>
<td></td>
<td>229</td>
<td>Dog urine</td>
<td>6</td>
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<td>236</td>
<td>Dog urine</td>
<td>6.5</td>
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<tr>
<td></td>
<td>237</td>
<td>Dog urine</td>
<td>2.8, 4.5 and 11.5</td>
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<tr>
<td></td>
<td>238</td>
<td>Dog prepuce</td>
<td>2.8 and 4.5</td>
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<tr>
<td></td>
<td>240</td>
<td>Dog faeces</td>
<td>2</td>
<td></td>
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<td></td>
<td>241</td>
<td>Dog urine</td>
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<td>Dog faeces</td>
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<td>246</td>
<td>Dog uterus</td>
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<td>247</td>
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<td>254</td>
<td>Dog vagina</td>
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<td>260</td>
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<td>11.5 and 2.8</td>
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<td>1297</td>
<td>Dog ear</td>
<td>1.7</td>
<td></td>
<td></td>
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<tr>
<td>*Proteus vulgaris</td>
<td>900</td>
<td>Cow milk</td>
<td>–</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1315</td>
<td>Horse</td>
<td>–</td>
<td></td>
<td></td>
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<tr>
<td>*Morganella morganii</td>
<td>911</td>
<td>Hedgehog nose</td>
<td>–</td>
<td></td>
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<tr>
<td></td>
<td>1316</td>
<td>Horse</td>
<td>–</td>
<td></td>
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<tr>
<td></td>
<td>1317</td>
<td>Cow organs</td>
<td>–</td>
<td></td>
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<tr>
<td>*Providencia rettgeri</td>
<td>1318</td>
<td>Dog urine</td>
<td>–</td>
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<tr>
<td></td>
<td>1319</td>
<td>Dog ear</td>
<td>–</td>
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</tbody>
</table>

*, no bands observed.

* Strain numbers from our collection are preceded by the abbreviation IVB for Institute for Veterinary Bacteriology.

† UCA bands obtained by hybridization with the EcoRI–PstI probe (about 700 bp) from IVB1433 (Fig. 1).

‡ Pmp-bands obtained by hybridization with the ClaI probe (648 bp) from IVB1430 (Fig. 1).

Pathogenic *E. coli* (UPEC) (Garcia et al., 1988a, b; Marklund et al., 1992; Kuehn et al., 1994). In human UPEC, P-fimbriae, which bind to glycosphingolipids of uroepithelial cells, are the most prevalent type of fimbriae (Garcia et al., 1988a; Johnson, 1991; Lund et al., 1988). They are encoded by the *pap* (pyelonephritis-associated pil) operon of which the *papA* gene encodes the major fimbrial subunit, whereas the *papG* gene encodes a receptor-binding protein, located at the tip of the fimbriae (Hultgren & Normark, 1991; Hal Jones et al., 1992).

Several serological variants of P-fimbriae exist (Denich et al., 1991; Garcia et al., 1992; Kusters & Gaastra, 1994). Human and canine UPEC isolates have different binding specificity, which can be attributed to differences in the PapG protein (Garcia et al., 1988a, b; Marklund et al., 1992).

A similar host-specific adhesion could not be demonstrated for uropathogenic *P. mirabilis* (UPPM) strains (Garcia et al., 1988b). UPPM strains generally produce
fimbriae, but these fimbriae have been studied far less than those of UPEC. Thus far, the existence of four fimbrial structures has been reported for human UPPM. Wray et al. (1986) isolated a fimbrial uroepithelial cell adhesin (UCA) and determined the N-terminal amino acid sequence of the major fimbrial subunit. Subsequently, Bahrani and co-workers identified mannose-resistant/Proteus-like (MR/P) fimbriae (Bahrani & Mobley, 1993) and P. mirabilis fimbriae (Bahrani et al., 1993). The nucleotide sequences of the major fimbrial subunit genes, mrpA and pmfA, were determined. In addition, the total mrp operon was sequenced (Bahrani & Mobley, 1994).

Finally, Massad et al. (1994) identified a new ambient-temperature fimbria (ATF) in UPPM. The N-terminal amino acid sequence of ATF did not show significant similarity to that of any previously described fimbrial protein. Several authors (Bahrani & Mobley, 1993; Duguid & Old, 1980; Mobley & Chippendale, 1990; Old & Adegbola, 1982; Silverblatt, 1974) demonstrated that different kinds of fimbriae may be found in the same strain of P. mirabilis. Similarly, UTI-related E. coli strains have been shown to express as many as four types of fimbriae (Rhen, 1985).

In this study, the isolation of two genes that encode different fimbrial subunits in P. mirabilis isolates from canine UTI is reported. The nucleotide sequences of the genes were determined and the deduced amino acid sequences have been compared with the major subunit sequences of other fimbriae from uropathogenic isolates.

METHODS

Bacterial strains and plasmids. The strains of P. mirabilis, P. vulgaris, Morganella morgani and Providencia rettgeri used in this study were obtained from the Veterinary Microbiological Diagnostic Centre of Utrecht University, The Netherlands, and are listed in Table 1.

E. coli PC2495 (Phabagen Collection, Department of Molecular Cell Biology, Utrecht University, The Netherlands), a bsdS recA derivative of E. coli JM101 was used as host for pBluescript (Stratagene) and its derivatives.

Isolation and analysis of fimbriae. The purification of fimbriae, assay of the purity of these preparations and total cell protein profiles by SDS-PAGE and Western blotting were performed as described by Mooli et al. (1987). The N-terminal amino acid sequence of purified fimbrial protein was determined at the Gas Phase Sequenator Facility (Department of Medical Biochemistry, State University of Leiden, The Netherlands). The instrument used was an Applied Biosystems Model 470A Protein Sequencer, equipped with an on-line Model 120A PTH Analyser. Electron microscopy of purified fimbriae preparations was done as described by Garcia et al. (1988a).

Recombinant DNA techniques. Chromosomal DNA was isolated as described by Garcia et al. (1988a). DNA restriction fragments were prepared by digestion of chromosomal DNA with the appropriate restriction endonucleases and the desired fragment was isolated after separation by electrophoresis of the digestion mixture in 1% (w/v) agarose gel and extraction from the agarose using the Geneclean kit (BIO 101). Ligation of DNA fragments into pBluescript was performed as described by Gastr & Hansen (1984). Transformation of CaCl₂-treated PC2495 cells was carried out according to standard procedures (Sambrook et al., 1989). The strategy for cloning and subcloning of DNA fragments from P. mirabilis chromosomal DNA is shown in Fig. 1. pBluescript derivatives containing restriction fragments derived from P. mirabilis chromosomal DNA were isolated using the Qiagen plasmid kit as specified by the manufacturer.

PCR. After transformation, colonies containing cloned chromosomal DNA fragments from P. mirabilis in pBluescript were screened by PCR. DNA amplification with Taq polymerase in Taq polymerase buffer (Promega) was performed with the primers T3 (5'TATTAACCTCAGCATAA 3') and T7 (3' GATATCCTCAGCATAA 5') flanking the insert in pBluescript. Colonies were aseptically transferred to a 100 μl reaction mix for amplification. PCR reactions were performed in an Omnigene Temperature Cycler (Hybaid) with 35 cycles of 1 min at 95 °C, 1 min at 48 °C and 1 min at 72 °C. After the final cycle, the mixture was incubated for 10 min at 72 °C to complete the last polymerase reaction.

**Fig. 1.** Physical maps of the cloned and subcloned chromosomal DNA fragments containing the indicated major subunit gene or parts thereof. The positions of both genes are indicated by open boxes, preceded by the signal sequences in black boxes. The arrows under each clone indicate the sequencing strategy. Restriction sites: C, CiaI; D, DraI; E, EcoRI; H, HindIII; P, PstI.
Southern blotting and DNA hybridization. Chromosomal DNA digests and the PCR products were run in a 1% agarose gel and transferred to nitrocellulose filters (BA/85, Schleicher & Schuell) by Southern blotting using standard procedures (Sambrook et al., 1989). Screening for cloned DNA fragments larger than 3 kb was done by colony blotting. For that purpose, colonies were transferred in duplicate to LB agar plates supplemented with 100 μg ampicillin ml⁻¹. After growth, one of the plates was used for blotting colonies onto nitrocellulose filters (BA/85). Colonies were lysed and DNA was immobilized on the plates was used for blotting colonies onto nitrocellulose (Sambrook et al., 1989). Oligonucleotide probes ( < 30 bp) were end-labelled with [γ-32P]ATP, whereas larger fragments ( > 200 bp) were labelled with [α-32P]ATP using a random primer labelling kit (Promega). Before prehybridization (2 h), filters were wetted in 5 x SSPE (Sambrook et al., 1989). Prehybridization and hybridization with larger probes were carried out at 42 °C, subsequent washing being at 50 °C. Hybridization and washings with oligonucleotide probes were done at 37 °C. After hybridization (16 h) and washing, filters were dried and mounted for autoradiography.

Nucleotide sequence analysis. The sequence of the cloned DNA fragments was determined with an Automated Laser Fluorescent DNA sequencer (Pharmacia). In addition to the fluorescein isothiocyanate (FITC)-labelled SK, KS and T7 Bluescript primers, FITC-labelled internal primers (Pharmacia) were used to determine ucaA and pmpA sequences.

Computer analysis. Nucleotide sequences were analysed using the PC/GENE program (release 6.70, Genofit). Similar amino acid sequences were located by searching all available databases with the BLAST programs (Altschul et al., 1990) as provided by the National Center for Biotechnology Information (NCBI) BLAST E-mail Server. Sequences were retrieved from the NCBI databases and aligned with MULTALIGN (Corpet, 1988). A phylogenetic tree (Fitch & Margoliash, 1967) was derived from the amino acid difference matrix calculated from these alignments with PHYLIP (Phylogeny Inference Package) version 3.5c (Felsenstein, 1989).

RESULTS

UcaA fimbrial subunit

Fimbriae from the dog UTI-associated P. mirabilis strains IVB219 and IVB292 were isolated and the sequence of the first 25 amino acid residues of the major subunit protein was determined (Fig. 2). The sequence appeared identical to the N-terminal amino acid sequence of the UCA fimbriae major subunit protein from human P. mirabilis isolates, reported by Wray et al. (1986). Upon SDS-PAGE, the purified fimbrial protein revealed only one band corresponding to a mass of approximately 17.5 kDa. In electron microscopy, we only observed thin fimbriae with a diameter of 4 nm. These data further confirmed the resemblance to the results of Wray et al. (1986).

We designed an oligonucleotide probe, B92–96, based on the sequence of amino acids 12–20 of the N-terminal amino acid sequence of the UCA major subunit protein. The neutral base inosine (I) was used in third positions of three- or fourfold ambiguity (Fig. 2). This probe hybridized with a 2 kb fragment in a PstI–HindIII digest of chromosomal DNA from strain IVB247 which eventually resulted in strain IVB1433 (Fig. 1). Subsequently, we isolated an EcoRI fragment of 2.7 kb from the chromosomal DNA of strain IVB247 by means of a DraI–PstI fragment from the 2 kb fragment as a probe. Following the strategy as indicated in Fig. 1, we sequenced the gene for the UCA major subunit. Analysis of the derived nucleotide sequence yielded a 540 bp ORF encoding a polypeptide of 180 amino acids, including a signal peptide of 22 amino acids (Fig. 3). The molecular mass of the processed polypeptide of 158 amino acids was calculated to be 16724 Da. The first 25 amino acids of this mature subunit protein were identical to the N-terminal amino acid sequence of the UCA fimbrial subunit as determined by Wray et al. (1986) for fimbriae from human P. mirabilis isolates.

PmpA-like fimbrial subunit PmpA

A second oligonucleotide probe was designed on the basis of amino acids 2–11 of the N-terminal amino acid sequence of the UCA major subunit protein. To minimize the degeneracy of this probe, we took into account the homology with the K99 major subunit that is found particularly in this region (Roosendaal et al., 1984; Wray et al., 1986). This probe 220 (Fig. 2) was used to screen PstI–HindIII-digested chromosomal DNA from P. mirabilis strains IVB219 and IVB292 in Southern hybridization. A hybridizing fragment of approximately 2.3 kb from strain IVB219 was cloned into pBluescript and yielded strain IVB1425 (Fig. 1). In the strategy for cloning and sequencing (Fig. 1), the subcloned ClaI fragment in plIVB1430 was used as a probe to isolate the 5 kb HindIII fragment for subsequent cloning into pIVB1431.

DNA sequence analysis revealed a 549 bp ORF. Downstream of the ORF, at positions 1058–1080, a potential inverted repeat was found (Fig. 4). Computer

| Tyr | Asp | Gly | Thr | Ile | Thr | Phe | Thr | Gly | Lys | Val | Val | Ala | Gln | Thr | Cys | Ser | Val | Asn | Thr | Asn | Asp | Lys | Asn | Leu |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     |     | GAT | ACT | ATT | ACC | TGG | TAC | ACT | GCC | ACA | AAT | GTA | GTT | GCT | CAA | AGT | TTC | TCT | GTC | GTI | AAT | GAC |

probe 220

probe B92–96

Fig. 2. Alignment of the UCA N-terminal amino acid sequence and the deduced oligonucleotide probes 220 and B92–96 that were used for the isolation of the pmpA and ucaA, respectively.
Analysis of UcaA and PmpA in Proteus strains

A total of 33 Proteus strains and Proteus-related strains, listed in Table 1, were screened for the occurrence of both the ucaA gene and the pmpA gene. Of 26 P. mirabilis strains, 22 were positive for the ucaA probe, whereas 24 strains were positive for the pmpA probe. Sixteen of these P. mirabilis strains had been isolated from canine urine and all 16 strains were positive for both probes, except strain IVB227 which hybridized only with the pmpA probe. P. vulgaris or other Proteus-related species are isolated at a much lower frequency from patient materials (e.g., urine, feces, organs). None of the seven isolates, other than P. mirabilis, reacted with the ucaA probe or with the pmpA probe.

Comparison of UcaA and PmpA amino acid sequences with other fimbrin sequences

The deduced amino acid sequence of the UcaA major fimbrial subunit closely resembled both the F17 (Lintemans et al., 1988) and the F111 (Lintemans, 1990) structural fimbral proteins (Fig. 5). The unprocessed UcaA protein appeared to share 56.1 % amino acid identity with the F17 major fimbrial subunit as well as 58.3 % identity with the F111 major subunit. However, comparison of the mature UcaA amino acid sequence with that of the PmpA major fimbrial subunit revealed only 18.4 % identity. The identical amino acid residues were mainly found in the N- and C-terminal regions. It was especially remarkable that the successive PmpA N-terminal residues 4-13 were identical to residues 2-11 of UcaA.

Alignment of all the known sequences of the major fimbrial subunit proteins from UTI-related isolates of P. mirabilis and E. coli, as well as of homologous fimbrial sequences from other sources yielded a phylogenetic tree (Fig. 6) in which all the different Pap fimbrins of UPEC fell within one branch. The aligned amino acid sequence of PmpA showed an identity ranging from 40.8 to 43.7 % with the F17 major fimbrial subunit as well as 58.3 % identity with the F111 major subunit. However, comparison of the mature UcaA amino acid sequence with that of the PmpA major fimbrial subunit revealed only 18.4 % identity. The identical amino acid residues were mainly found in the N- and C-terminal regions. It was especially remarkable that the successive PmpA N-terminal residues 4-13 were identical to residues 2-11 of UcaA.

**DISCUSSION**

In this study, UCA fimbriae have been demonstrated in and isolated from a number of canine P. mirabilis strains. Since the N-terminal amino acid sequence of these fimbriae appeared identical to the N-terminal amino acid sequence of UCA fimbriae from human P. mirabilis isolates (Wray et al., 1986), it was our prime goal to identify, isolate and sequence the major fimbrial subunit gene ucaA from a P. mirabilis strain originating from a dog with UTI. We inadvertently first isolated a P-fimbriae-like major subunit gene, pmpA, merely because the amino acid...
I. G. W. BIJLSMA and OTHERS

The amino acid sequence of the UcaA major subunit from the dog-uropathogenic P. mirabilis strain IVB247 compared to the amino acid sequences of the fimbrial subunits F17A (Lintermans et al., 1988) and F111A (Lintermans, 1990) from bovine enterotoxigenic E. coli.

sequence chosen for the design of an oligonucleotide probe was identical in both the UcaA and PmpA polypeptides. Wray et al. (1986) had already demonstrated significant homology of UcaA to the K99 N-terminal sequence, particularly among the first 10 residues (60% identity). Therefore, the K99 nucleotide sequence (Roosendaal et al., 1984) was used to design probe 220, especially in third positions of three- or fourfold ambiguities. Yet, probe 220 appeared to match the pmpA nucleotide sequence much better than the ucaA sequence (Fig. 4). Bahrami et al. (1993) had a comparable situation when they identified pmfA using a probe also designed from amino acid residues (19-24) of the same UcaA N-terminal sequence. We designed a new probe on the basis of the subsequent amino acids (12-20) of the UcaA N-terminal sequence and eventually succeeded in the isolation of the pmpA gene. The occurrence of UcaA fimbriae has now been demonstrated both in canine isolates of P. mirabilis (this paper) and in human isolates (Wray et al., 1986). The N-terminal amino acid sequences of the major subunits from the human and canine isolates appeared identical. Moreover, the amino acid composition derived from the translated ucaA gene is an exact match for the 25 amino acid residues of the UcaA N-terminal sequence. We designed a new probe on the basis of the subsequent amino acids (12-20) of the UcaA N-terminal sequence and eventually succeeded in the isolation of the ucaA gene. The occurrence of UcaA fimbriae has now been demonstrated both in canine isolates of P. mirabilis (this paper) and in human isolates (Wray et al., 1986). The N-terminal amino acid sequences of the major subunits from the human and canine isolates appeared identical. Moreover, the amino acid composition derived from the translated ucaA gene is an exact match for the 25 amino acid residues of the UcaA N-terminal sequence.

Though we did not isolate Pmp fimbriae, there is also strong evidence for the occurrence of Pmp fimbriae on canine UPPM isolates. Bahrami et al. (1991) isolated a mannose-resistant/Proteus-like (MR/P) type of fimbriae from a human UPPM strain and determined the N-terminal amino acid sequence of the major subunit which was designated MrpA. The N-terminal amino acid sequence of this MrpA subunit appeared identical to the first 20 amino acids of the processed polypeptide that we obtained after translation of the pmpA nucleotide se-
sequence. However, in a subsequent paper (Bahrani & Mobley, 1993), the nucleotide sequence for a different major fimbrial subunit was designated as mrpA and the authors did not mention the previous MrpA sequence. For this reason and because of its P-fimbrial nature, the nucleotide sequence that we determined was designated PmpA.

The PmpA protein in turn provided a close relationship with the major subunits of the P-fimbriae from UPEC. The close relationship of the F11 and F165 major fimbrial subunits (96% identity) is also interesting in this context, since F165 is associated with E. coli septicemia in piglets (Harel et al., 1992). It might therefore be speculated that the UCA type or the Pmp type of fimbriae on P. mirabilis isolates can be used for adhesion in the intestinal tract rather than in the urinary tract, in spite of the fact that it has been demonstrated that UCA fimbriae can mediate adhesion to uroepithelial cells (Wray et al., 1986). Specific adhesion to uroepithelial cells should then be mediated by one of the other types of fimbriae produced by P. mirabilis isolates. This speculation is supported by the fact that most UTI-related P. mirabilis strains appeared to have genetic information for at least two different major subunits, UcaA and PmpA (Table 1), and by the observation that faecal samples of dogs with recurrent UTI due to P. mirabilis contained 10^4 Proteus cells per gram, whereas faecal samples of control dogs were negative (Gaast et al., 1995). A role for P-fimbriae of UPEC in the colonization of the large intestine has likewise been suggested (Wold et al., 1988, 1992; Svanborg et al., 1994).

P-fimbriae-like sequences have been found in E. coli, P. mirabilis and Serratia marcescens strains isolated from UTI (Krogfelt, 1991; Kusters & Gaasra, 1994; Mizuno et al., 1988). The MrpA protein appeared to share a high amino acid sequence identity with the SmfA subunit from a uropathogenic S. marcescens isolate and with the PapA subunit in fimbriae from UPEC (Bahrani & Mobley, 1993). So far, P. mirabilis is the only Proteus species that has shown to have the genetic information for the production of this type of fimbria. The genetic information for these fimbriae could not be demonstrated in other Proteus-related species (Table 1). Bahrani et al. (1993) obtained similar results for human P. mirabilis isolates. Therefore, we think that P. mirabilis has acquired the genes for these fimbriae recently in evolution by horizontal gene transfer, most probably from E. coli. Also, the fact that UPPM strains in contrast to UPEC strains do not exhibit host specificity in adhesion (Garcia et al., 1988b), is in favour of this hypothesis.

Of the four major fimbrial subunit proteins of P. mirabilis for which the amino acid sequence is known, i.e. UcaA, MrpA, PfmA and PmpA, PmpA shows the closest relationship to the E. coli P-fimbrial major subunits. The evolutionary distance between the PfmA, MrpA and PmpA proteins is much larger. The similarity between PmpA and one of the P-fimbrial F serotypes is not higher than with any of the others, which indicates that P. mirabilis must have obtained the gene encoding this protein before the divergence of the various F serotypes in E. coli took place. On the other hand, K99 must have branched off earlier from the other P. mirabilis fimbrins on the phylogenetic tree (Fig. 6).

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