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Construction of an MR/P Fimbrial Mutant of *Proteus mirabilis*: Role in Virulence in a Mouse Model of Ascending Urinary Tract Infection

FARAH K. BAHRANI, GEORGE MASSAD, C. VIRGINIA LOCKATELL, DAVID E. JOHNSON, ROBERT G. RUSSELL, JOHN W. WARREN, AND HARRY L. T. MOBLEY*

Division of Infectious Diseases, Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201

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***Proteus mirabilis*, a cause of acute pyelonephritis, produces at least four types of fimbriae, including MR/P (mannose-resistant/*Proteus*-like) fimbriae. To investigate the contribution of MR/P fimbriae to colonization of the urinary tract, we constructed an MR/P fimbrial mutant by allelic exchange. A 4.2-kb *Bam*HI fragment carrying the *mrpA* gene was subcloned into a mobilizable plasmid, pSUP202. A 1.3-kb Kan^r cassette was inserted into the *mrpA* open reading frame, and the construct was transferred to the parent *P. mirabilis* strain by conjugation. Following passage on nonselective medium, 1 of 500 transconjugants screened was found to have undergone allelic exchange as demonstrated by Southern blot. Colony immunoblot, Western immunoblot, and immunogold labeling with a monoclonal antibody to MR/P fimbriae revealed that MrpA was not expressed. Complementation with cloned *mrpA* restored MR/P expression as shown by hemagglutination, Western blot, and immunogold electron microscopy. To assess virulence, we challenged 40 CBA mice transurethrally with 10^7 CFU of wild-type or mutant strains. After 1 week, geometric means of $\log_{10}$ CFU per milliliter of urine or per gram of bladder or kidney for the wild-type and mutant strains were as follows: urine, 7.79 (wild type) versus 7.02 (mutant) ($P = 0.035$); bladder, 6.22 versus 4.78 ($P = 0.019$); left kidney, 5.02 versus 3.31 ($P = 0.009$); and right kidney, 5.28 versus 4.46 ($P = 0.039$). Mice challenged with the wild-type strain showed significantly more severe renal damage than did mice challenged with the MR/P-negative mutant ($P = 0.007$). We conclude that MR/P fimbriae contribute significantly to colonization of the urinary tract and increase the risk of development of acute pyelonephritis.**

Following the development of bacteriuria, *Proteus mirabilis*, a common uropathogen, can cause serious complications including kidney stone formation, acute pyelonephritis, and bacteremia (9, 29). Several virulence determinants, including urease (13, 17), hemolysin (17, 18, 24, 25, 34), flagella (23), fimbriae (26, 30, 31, 33), and immunoglobulin A protease (28), have been demonstrated for this species. Thus far, several different hemagglutinins and fimbriae, including mannose-resistant/*Proteus*-like (MR/P) fimbriae (1, 22), mannose-resistant/*Klebsiella*-like (MR/K) fimbriae (1, 22), *P. mirabilis* fimbriae (PMF) (2), uroepithelial cell adhesin (38), and the ambient-temperature fimbriae (ATF) (4), have been described for this organism.

A number of reports suggest that MR/P fimbriae are involved in development of pyelonephritis and that these structures mediate adherence of *P. mirabilis* to uroepithelial cells. We used hemagglutination assays to show that MR/P fimbriae are expressed by most pyelonephritis-associated strains as the only hemagglutinin type (17), in contrast to strains isolated from feces of healthy women or from patients with catheter-associated bacteriuria, which produce an additional hemagglutinin. Sareneva et al. (26) demonstrated that in vitro binding of *P. mirabilis* to tubular epithelial cells of frozen sections of human kidney was inhibited by an antibody to MR/P fimbriae. In an in vivo challenge, it was shown that an antibody response to a fimbria which hemagglutinates chicken erythrocytes in the presence of mannose (the putative phenotype of MR/P fimbriae) protected animals against urinary tract infection (UTI) by *P. mirabilis* strains (14). In a separate study, we have also demonstrated that MR/P fimbriae induced antibody response in chronically infected mice, indicating that these fimbriae are expressed during the course of UTI in experimentally infected mice and are immunogenic (3).

The genes encoding MR/P fimbriae of *P. mirabilis* have been cloned in *Escherichia coli* (5) and sequenced (6) in our laboratory. *mrpA*, the gene encoding the major fimbrial subunit, is a 525-bp open reading frame which predicts a polypeptide of 175 amino acids including a 23-amino-acid signal peptide (5). The DNA sequence of the *mrp* gene cluster carries seven other genes (6) similar to those of other fimbrial operons including the *pap* operon of uropathogenic *E. coli* (35).

The purpose of this study was to assess the contribution of MR/P fimbriae of *P. mirabilis* to colonization of the urinary tract and development of acute pyelonephritis. We constructed an isogenic MR/P fimbrial mutant and compared its ability with that of wild-type strains to colonize and cause histological damage in the well-established CBA mouse model of ascending UTI.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *P. mirabilis* HI4320 was isolated from the urine of a patient with catheter-associated bacteriuria (>10^5 CFU/ml of urine) (20, 37). *P. mirabilis* strains were passaged three times in nutrient broth statically for 48 h at 37°C, conditions that favor the expression of MR/P fimbriae. *E. coli* HB101, grown in Luria broth or agar and supplemented with appropriate antibiotics, was used as a host

* Corresponding author. Mailing address: Division of Infectious Diseases, University of Maryland School of Medicine, 10 S. Pine St., Baltimore, MD 21201. Phone: (410) 706-7560. Fax: (410) 706-8700.

for transformation. Bacteria were stored at −70°C in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 15% glycerol.

Recombinant DNA methods. Chromosomal DNA was isolated by the method of Maniatis et al. (15). Plasmid DNA was isolated by using Qiagen columns as specified by the manufacturer (Qiagen, Inc.). Restriction fragments were labeled by random priming with [α-^{32}P]ATP (specific activity, 3,000 Ci/mmol). Colony blot hybridization, Southern blot analysis, transformation, and other genetic techniques were performed by standard techniques (15).

Western blot analysis. Whole-cell preparations were denatured with 10% trichloroacetic acid (necessary to denature MR/P fimbriae [3]), solubilized in sodium dodecyl sulfate (SDS)-gel sample buffer, subjected to SDS-polyacrylamide gel electrophoresis (PAGE) (15% polyacrylamide), and transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore) by the method of Towbin et al. (36). Western immunoblots were developed with a polyclonal antiserum raised against purified MR/P fimbriae (3).

Immunogold electron microscopy. Bacteria were grown under conditions optimal for production of MR/P fimbriae (see above). Immunogold labeling was performed by a modification of the method of Faulk and Taylor (8). Bacteria were pelleted and washed, and a drop of bacterial suspension was placed on a Formvar-coated grid (Electron Microscopy Sciences, Fort Washington, Pa.) for 2 min. Excess liquid was wiped, off and the specimen was air dried. The grid was incubated on a drop of a 1:100 dilution of a monoclonal antibody to MR/P fimbriae for 30 min. After the grid was washed three times with phosphate-buffered saline (PBS) containing 1% bovine serum albumin, a drop of a 1:9 dilution of a 5-nm-gold-labeled goat anti-mouse immunoglobulin G (AuroProbe EM protein GG5; Amersham Corp., Amersham, England) was added for 45 min and the grids were washed three times. The grids were negatively stained with 1% sodium phosphotungstic acid (pH 6.8) and examined by transmission electron microscopy.

Hemagglutination. Bacteria were grown under the conditions optimal for production of MR/P fimbriae (see above). Bacterial pellets were resuspended in PBS to approximately 10^9 CFU/ml and then mixed with an equal volume of a 3% (vol/vol) suspension of chicken erythrocytes in the presence or absence of 50 mM mannose. Rapid clumping of the bacteria in the presence and absence of mannose indicates the presence of MR/P fimbriae.

Other assays. Hemolysin activity (17) and uroepithelial cell adherence (19) were determined as previously described.

Mouse model of ascending UTI. A modification (12) of the mouse model of Hagberg et al. (10) was used. Female mice (20 to 22 g, 6 to 8 weeks old; Jackson Laboratory, Bar Harbor, Maine) were used after they had been tested for the absence of bacteriuria. Mice, anesthetized with methoxyflurane, were inoculated with 10^7 CFU of bacteria (suspended in 0.05 ml of PBS) through a sterile polyethylene catheter (outer diameter, 0.61 mm) inserted into the bladder through the urethra. One week later, the mice were sacrificed by administration of an overdose of methoxyflurane. Urine was collected, and the bladder and both kidneys were removed aseptically and weighed. The urine, the bladder, and half of each kidney were quantitatively cultured, and viable counts were determined as CFU per milliliter of urine or CFU per gram of tissue.

For evaluation of renal histopathology in mice at 1 week postchallenge, kidneys were cut longitudinally and half of each kidney was fixed in 10% neutral buffered formalin. The kidneys were embedded in paraffin, and sections were stained with hematoxylin and eosin. The severity of pathologic changes in the kidneys of mice challenged with the wild type or mutant was determined by using a semiquantitative score of the severity of pyelitis or pyelonephritis (1+, mild pyelitis with individual uroepithelial cells lining the pelvic cavity undergoing degeneration or necrosis and small to moderate numbers of neutrophils in the pelvic cavity; 2+, moderate pyelitis with diffuse damage to the uroepithelium but with no disruption of the uroepithelium, and small numbers of neutrophils in the adjacent parenchyma and small to moderate numbers of neutrophils in the pelvic cavity; 3+, moderate pyelitis with diffuse thickening of the uroepithelium accompanied by prominent infiltration of neutrophils and lymphocytes within and adjacent to the uroepithelium, with inflammation extending into the parenchyma immediately adjacent to the pelvic cavity and with neutrophil casts in the lumen of affected tubules and moderate numbers of neutrophils in the pelvic cavity; 4+, pyelonephritis in which the inflammatory cell infiltration is composed of moderate to large numbers of neutrophils and mononuclear cells in the interstitium of the medulla and cortex with damage to tubules in the affected area, which may be localized or involve up to 30% of the medulla and cortex; 5+, severe pyelonephritis with similar inflammatory cell infiltration and damage which is more extensive and involves >30% of the parenchyma). The investigator was blinded to the identification of slides when reading the histopathologic test results. The kidney from each mouse with the most pronounced changes was used for assigning the score of renal pathologic changes for individual mice.

Statistical analysis of the bacteriologic results (CFU per milliliter or per gram) and comparison of pathology scores of mice challenged with the wild type and the mutant were conducted by using Mann-Whitney nonparametric analysis. Animal protocols were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee.

RESULTS

Construction of the *mrp* mutation. Cosmid clone pMRP101 carries the *mrp* gene cluster. A 4.2-kb *Bam*HI fragment within this cosmid, which contains the *mrpA* open reading frame between approximately equal lengths of DNA, was cloned into the mobilizable plasmid pSUP202 (Amp^r) (32). The resulting construct, pGUG4.2, carries *mrpA* on the 4.2-kb *Bam*HI fragment (Fig. 1) flanked by two other genes, *mrpI* (upstream) and *mrpB* (downstream), a potential regulatory and anchor gene, respectively. To mutate *mrpA*, pGUG4.2 was partially digested with *Pst*I and ligated with a 1.28-kb *Pst*I fragment carrying *aphA*, which encodes kanamycin resistance (GeneBlock; Pharmacia). An Amp^r Kan^r clone, found by restriction digestion to contain an insertion of the kanamycin cassette within *mrpA*, was designated pFKB1.28.

Construction of an MR/P fimbria-negative mutant. *E. coli* HB101(pFKB1.28) was conjugated with *P. mirabilis* HI4320 (Tet^r) in a triparental mating with *E. coli* HB101(pRK2013) as a helper for mobilization (Fig. 1). Transconjugants were selected on Luria agar containing tetracycline (20 μg/ml) and ampicillin (50 μg/ml). Twenty-five such colonies were passaged without antibiotic selection, which led to curing of the plasmid in some progeny. Of 500 colonies screened, 1 was ampicillin susceptible and kanamycin resistant, suggesting that marker exchange had occurred and that the plasmid had been lost.

Allelic exchange of the wild-type *mrpA* allele and *mrpA*::*aphA* was confirmed by Southern blot analysis. Chromosomal DNA from the mutant and the parent strain were digested with *Sal*I, which has sites on each side of *mrpA* but not within *aphA*

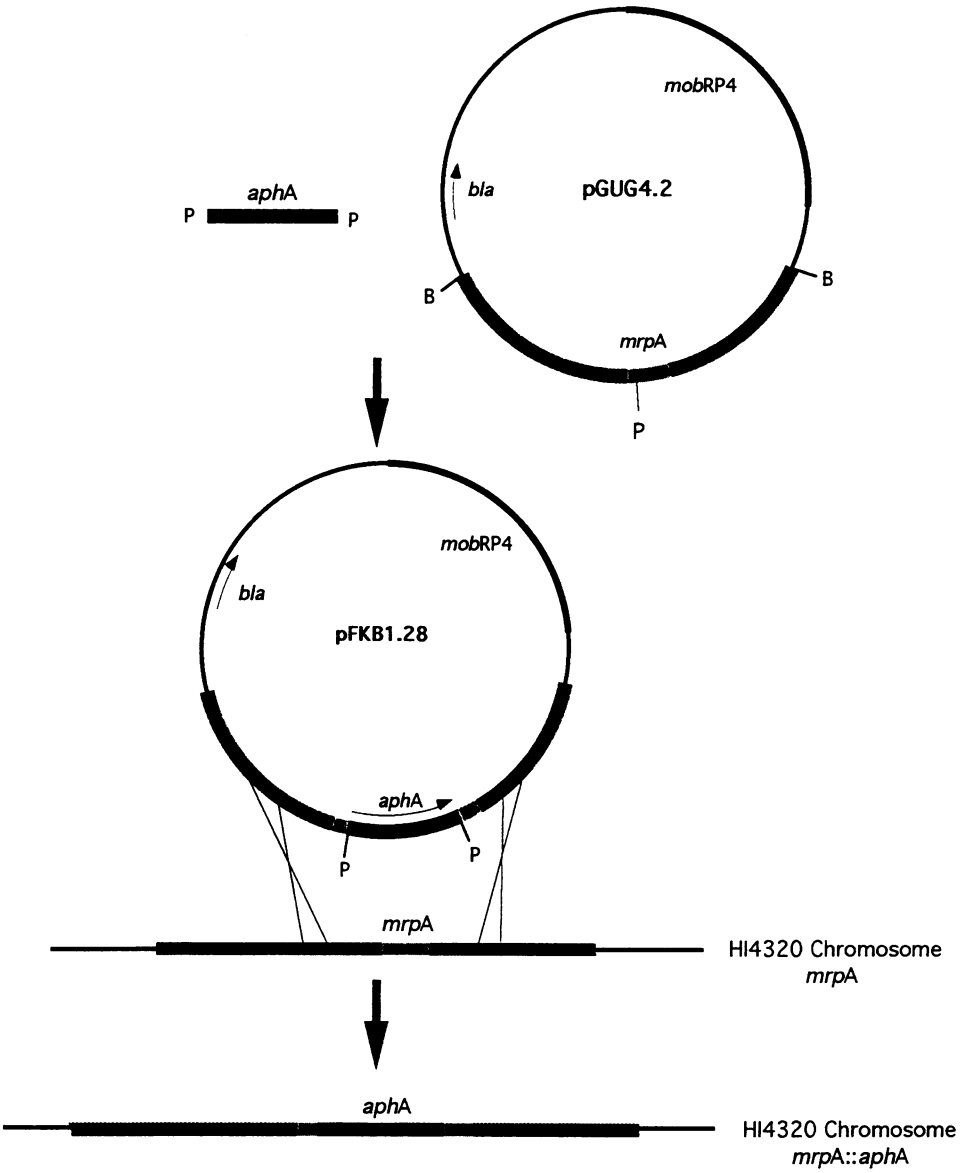

FIG. 1. Construction of the *mrpA* strain. A 4.2-kb *Bam*HI (B) fragment containing *mrpA* from cosmid clone pMRP101 was cloned into the *Bam*HI site of pSUP202 to make pGUG4.2. A 1.28-kb *Pst*I (P) fragment containing the *aphA* kanamycin resistance gene was cloned into the *Pst*I site within *mrpA* of pGUG4.2 to make pFKB1.28. pFKB1.28 was mobilized into *P. mirabilis* HI4320 for selection of cointegrates. Cointegrates were passaged on nonselective medium and then screened for kanamycin resistance and ampicillin susceptibility. One such colony, HI4320 *mrpA*::*aphA*, was isolated and characterized as an MR/P fimbrial mutant. *bla*, β-lactamase; *mob*RP4, mobilization genes.

(Fig. 1). By using a 277-bp *Pst*I-*Pvu*II fragment internal to *mrpA* as a probe, the Southern blot showed a band of 2.9 kb for the parent strain but a band of 4.2 kb (i.e., 1.3 kb larger, the size of the kanamycin resistance cassette) for the chromosomal DNA of the mutant strain (Fig. 2A). When the kanamycin cassette was used as a probe, a 4.2-kb band was observed only in the mutant strain (Fig. 2B) and had the same mobility as the band recognized by the *mrpA* probe in the mutant (Fig. 2A). No vector sequences were detected in either wild type or mutant when pSUP202 was used as a probe (data not shown). This mutant was designated *P. mirabilis* HI4320 *mrpA*::*aphA*.

Characterization of the mutant. Whole cells of the mutant did not react with a monoclonal antibody specific for MR/P fimbriae on colony immunoblots, whereas strain HI4320 reacted strongly (data not shown). On a Western blot of whole-cell protein, with a polyclonal antibody to MR/P fimbriae, MrpA, the major fimbrial subunit of apparent molecular mass of 16.4 kDa, was present in the parent strain but absent in the mutant strain (Fig. 3). In addition, what we believe to be high-molecular-weight multimers of MrpA are absent in the mutant. Furthermore, there was no immunogold labeling of the mutant strain with a monoclonal antibody to MR/P fimbriae (Fig. 4B) as observed by transmission electron microscopy, indicating that there was no detectable assembly of the structural subunits of MR/P fimbriae in the mutant strain. In contrast, fimbriae on the parent strain, HI4320, were labeled strongly with gold particles in this assay (Fig. 4A). Although the mutant strain does not express MR/P fimbriae, it does

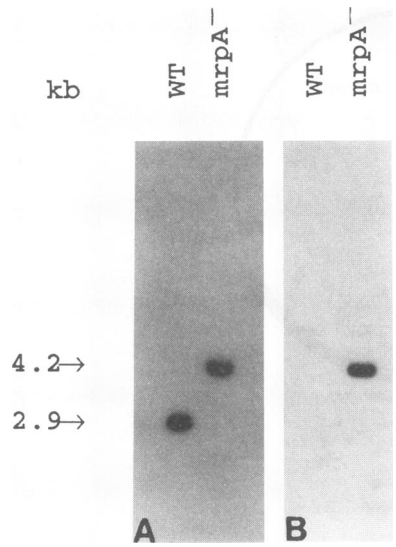

FIG. 2. Southern blot of chromosomal DNA from *P. mirabilis* HI4320 (lane WT [wild type]) and its MR/P fimbrial mutant (lane mrpA$^-$). DNA was digested with *Sal*I, electrophoresed, transferred to nitrocellulose, and hybridized with a 277-bp *Pst*I-*Pvu*II fragment internal to *mrpA* (A) or a 1.28-kb *Pst*I fragment carrying the *aphA* gene (kanamycin resistance cassette) (B).

produce at least two other types of fimbriae (Fig. 4B) as confirmed by SDS-PAGE of purified fimbrial preparations that show PMF fimbriae (2) and the ATF fimbriae (data not shown) (4).

MR/P hemagglutination is defined as agglutination of the erythrocytes of several animal species (including humans) in the presence or absence of mannose, with chicken erythrocytes

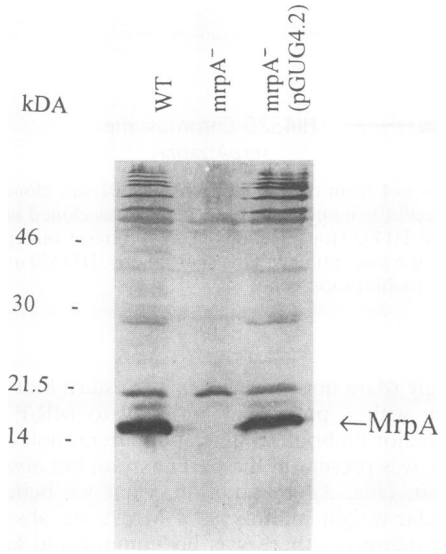

FIG. 3. Western blot of MR/P fimbriae. Whole-cell protein, denatured with 10% trichloroacetic acid, was electrophoresed on an SDS–15% polyacrylamide gel. Western blots were developed with polyclonal antiserum raised against purified MR/P fimbriae. Lanes: WT, wild-type strain HI4320; mrpA$^-$, HI4320 *mrpA*::*aphA*, the MR/P fimbrial mutant; mrpA$^-$(pGUG4.2), HI4320 *mrpA*::*aphA*(pGUG4.2), the MR/P fimbrial mutant complemented in *trans* with cloned *mrpA*.

giving the strongest reaction (22). Although there has been a very strong correlation between this hemagglutination pattern and the appearance of fimbriae, no direct evidence that MR/P fimbriae agglutinate erythrocytes in a mannose-resistant fashion has been provided. Insertional inactivation of *mrpA* within the *P. mirabilis* chromosome (i.e., strain HI4320 *mrpA*::*aphA*) resulted in both lack of expression of MR/P fimbriae and loss of MR/P hemagglutination (Fig. 5). These results demonstrate that *mrp* genes are responsible for this specific hemagglutination pattern.

To determine whether there were differences other than expression of MR/P fimbriae, we compared the mutant strain with the wild-type parent strain for a number of biochemical characteristics. The mutant and wild-type strains both produced urease, an established virulence factor, and had similar generation times of 54 and 50 min, respectively, in Luria broth. Identical hemolytic titers of 1:16 were measured for the mutant and the wild-type strains. In addition, both strains displayed swarming motility and had compatible Dienes swarming types (7) (data not shown). Also, no differences were found for any of the biochemical markers that are included in the Minitek numerical taxonomic system (BBL).

In a uroepithelial cell adherence assay, no significant difference in the ability to adhere to exfoliated uroepithelial cells between the wild type (34 ± 34 bacteria per uroepithelial cell; 70% of cells with ≥10 bacteria) and mutant (40 ± 27 bacteria per uroepithelial cell; 70% of cells with ≥10 bacteria) was found.

Complementation. Supplying *mrpA* in *trans* restored expression of the MR/P fimbriae. Plasmid pGUG4.2, which carries *mrpA*, was mobilized into strain HI4320 *mrpA*::*aphA* by triparental mating. The complemented mutant agglutinated chicken erythrocytes even more strongly than the wild-type strain did (Fig. 3C). In a Western blot developed with polyclonal antiserum to MR/P fimbriae, expression of MrpA was found to be restored (Fig. 4). Furthermore, the mutant strain carrying *mrpA* on a plasmid was shown to produce fimbriae that were immunogold labeled with antiserum to MR/P fimbriae when examined by transmission electron microscopy (Fig. 5C).

CBA mouse model of ascending UTI. To assess the contribution of MR/P fimbriae to colonization and virulence in the urinary tract, 20 CBA mice were challenged transurethrally with 10^7 CFU of wild-type or mutant strains. At 1 week postchallenge, the geometric means of $\log_{10}$ CFU per milliliter of urine or CFU per gram of bladder and CFU per gram of kidney for wild-type and mutant strains were as follows: urine, 7.79 (wild type) versus 7.02 (mutant) ($P = 0.035$); bladder, 6.22 versus 4.78 ($P = 0.019$); left kidney, 5.02 versus 3.31 ($P = 0.009$); right kidney, 5.28 versus 4.46 ($P = 0.039$) (Fig. 6). The MR/P fimbrial mutant colonized the urine, bladder, and kidneys in significantly smaller numbers than the wild-type strain did.

Renal histopathology. The mice challenged with the mutant strain showed significantly less severe renal damage than did the mice challenged with the wild-type strain ($P = 0.007$). Representative photomicrographs of renal abnormalities in mice challenged with the parent strain HI4320 (Fig. 7A) and the *mrpA* mutant (Fig. 7B) are shown. The mean pathology scores were 3.22 ± 0.44 for the parent strain and 2.30 ± 0.67 for the mutant. All mice challenged with wild-type strain HI4320 showed severe pyelitis (pathology score, 3+) with marked thickening of the uroepithelium, infiltration of moderate numbers of neutrophil inflammatory cells immediately beneath and within the epithelium, and infiltration of moderate numbers of neutrophils in the lumen of the pelvic cavity. Two mice showed pyelonephritis (pathology score, 4+). One

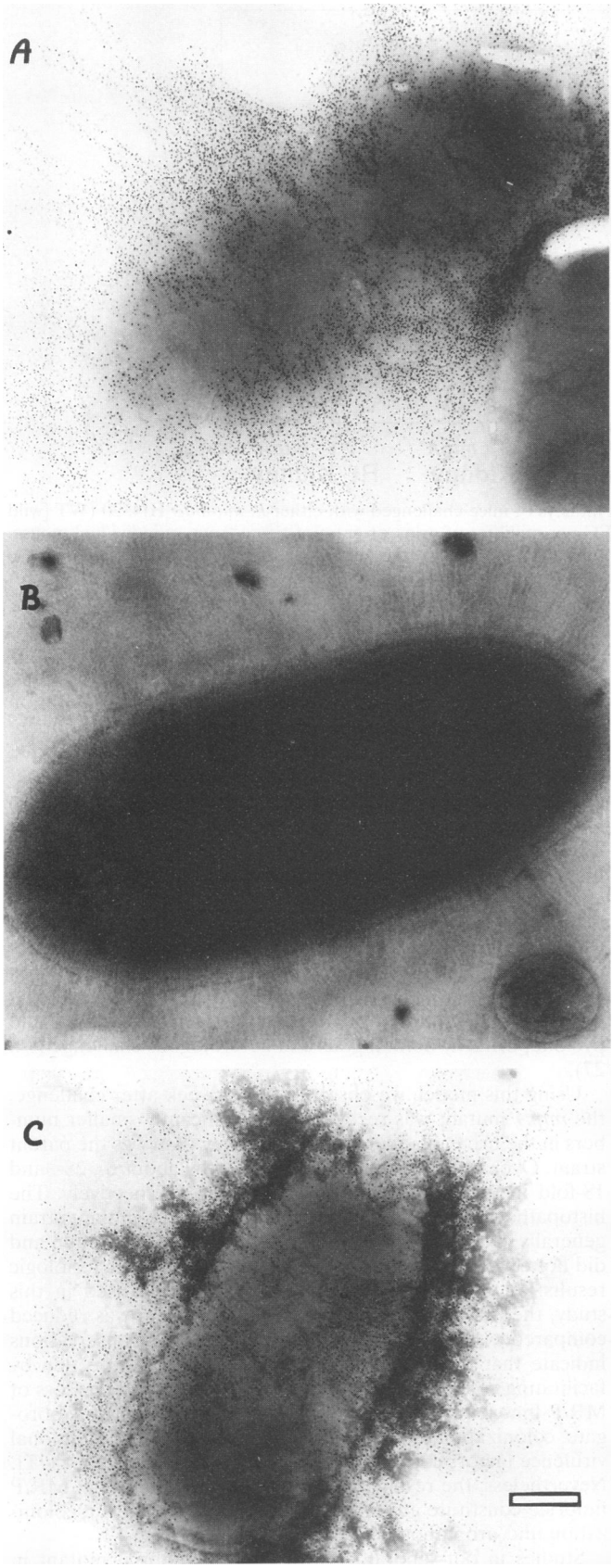

FIG. 4. Immunoelectron microscopy of *P. mirabilis* expressing MR/P fimbriae. Bacterial suspensions were incubated with monoclonal antibody to MR/P fimbriae and then with gold-labeled goat anti-mouse immunoglobulin G. Grids were negatively stained and observed by transmission electron microscopy. (A) Strain HI4320; (B) HI4320 *mrpA*::*aphA*, the MR/P fimbrial mutant; (C) HI4320 *mrpA*::*aphA* (pGUG4.2), the MR/P fimbrial mutant complemented in *trans* with cloned *mrpA*.

of these mice had a focal area of pyelonephritis, and the other showed extensive pyelonephritis, affecting approximately 25% of the kidney, with large numbers of neutrophils in the lumen of tubules and in the interstitium. Two mice had papillitis. By comparison, approximately half (n = 6) of the mice challenged with the mutant showed mild to moderate pyelitis (pathology scores, 1+ and 2+). The uroepithelium showed focal and diffuse epithelial damage, with localized areas of epithelial hyperplasia, but was not diffusely and markedly thickened. Infiltration of neutrophils was generally mild to moderate, and there were generally smaller numbers of neutrophils in the pelvic cavity. The remaining mice (n = 4) showed severe pyelitis, and two of four mice had papillitis (pathology score, 3+). None of the mice challenged with the mutant had pyelonephritis.

DISCUSSION

Construction of the *mrpA* fimbrial mutant demonstrates conclusively that the MR/P fimbriae is the MR/P hemagglutinin. Some years ago, Old and Adegbola (22) correlated the expression of a specific fimbrial structure observed by trans-

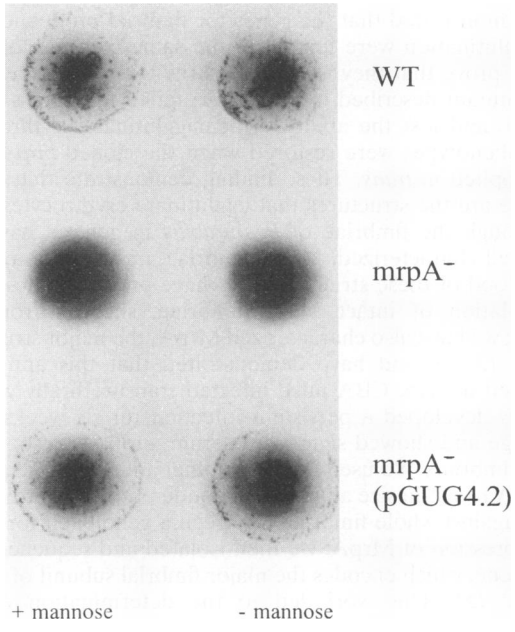

FIG. 5. Hemagglutination of *P. mirabilis* HI4320 and its *mrpA* mutant. A suspension of *P. mirabilis* was mixed with a 3% suspension of chicken erythrocytes in the presence and absence of 50 mM mannose. WT, wild-type parental strain HI4320; mrpA⁻, MR/P fimbrial mutant strain HI4320 *mrpA*::*aphA*; mrpA⁻(pGUG4.2), MR/P fimbrial mutant complemented in *trans* with cloned *mrpA*. Note the agglutination of erythrocytes by the wild-type strain and complemented mutant.

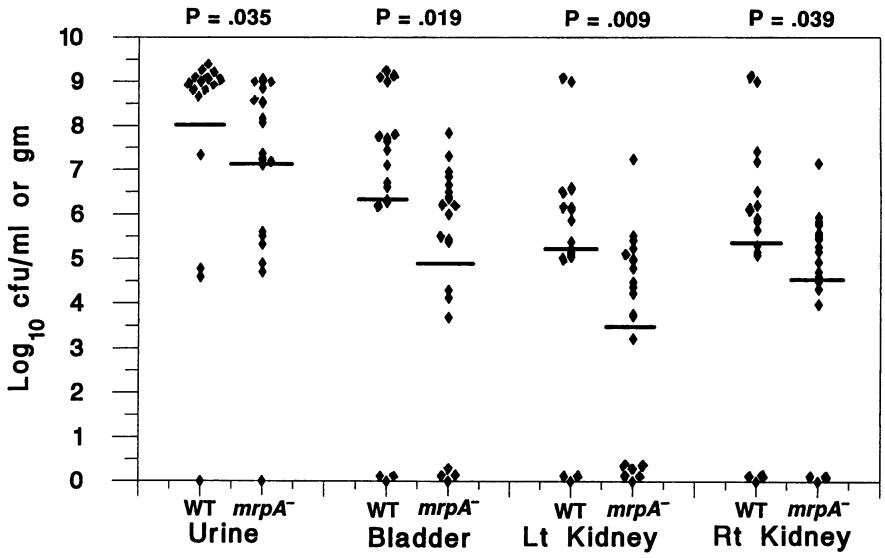

FIG. 6. Quantitative bacterial counts in urine specimens, bladders, and kidneys of mice challenged with either *P. mirabilis* HI4320 (WT [wild type]) or its MR/P fimbrial mutant (*mrpA*$^-$). Each diamond represents CFU per milliliter of urine or gram of tissue from an individual mouse. Horizontal lines represent the geometric mean of the colony counts. Diamonds near the *x* axis represent counts that are less than 100 CFU, the limit of detection in this assay. *P* values, shown at the top, were derived by using the Mann-Whitney test.

mission electron microscopy with the ability of *P. mirabilis* to agglutinate erythrocytes in a mannose-resistant fashion. Although the correlation was strong, the possibility that a hemagglutinin was coexpressed with this fimbria could not be ruled out. We previously isolated cosmid clone pMRP101 in our laboratory, and this clone conferred upon *E. coli* DH5α the ability to both produce fimbriae recognized by antiserum to native MR/P fimbriae and hemagglutinate erythrocytes (5). This demonstrated that the genes for fimbrial production and hemagglutination were linked on the same cosmid clone but did not prove that they were necessarily the same genes. The *mrpA* mutant described in this report failed to express MR/P fimbriae and lost the ability to hemagglutinate erythrocytes; these phenotypes were restored when the cloned *mrpA* gene was supplied in *trans*. These finding demonstrate that MR/P fimbriae are the structures that agglutinate erythrocytes.

Although the fimbriae of *P. mirabilis* in general have not been well characterized, MR/P fimbriae are perhaps the best understood of these structures. We have previously described the isolation of intact MR/P fimbriae, sheared from the surface; we have also characterized MrpA, the major structural subunit (3, 5), and have demonstrated that this antigen is expressed in vivo. CBA mice infected transurethrally with *P. mirabilis* developed a persistent infection for six weeks postchallenge and showed significant serum antibody response to MR/P fimbriae. We used the N-terminal amino acid sequence of MrpA to synthesize an oligonucleotide probe and antiserum raised against whole fimbriae to screen a gene bank for *mrpA* and expression of MrpA; we then isolated and sequenced the *mrpA* gene, which encodes the major fimbrial subunit of MR/P fimbriae (5). This work led to the determination of the complete nucleotide sequence of the *mrp* gene cluster, which predicted eight polypeptides, transcribed from *mrpIABCDEFG*, encoded by 7.3 kbp (6). The sequencing data revealed that the *mrp* gene cluster was organized in a fashion similar to that of the *pap* operon and other enteric fimbrial gene clusters.

The predicted genetic organization allowed us to evaluate the contribution of MR/P fimbriae of uropathogenic *P. mirabilis* to the ability of the bacterium to colonize the urinary tract. To investigate the role of MR/P fimbriae, we insertionally inactivated the *mrpA* gene sequence and generated a strain which was isogenic to the parent strain except for a mutation (kanamycin cassette insertion) in the *mrpA* locus. To compare the virulence of the *mrpA* mutant with that of its parent strain, we used a well-established animal model of ascending UTI that mimics the most common route of acquisition of UTI in humans. The CBA mouse model of ascending UTI, which involves female animals, has been used to differentiate pyelonephritogenic strains from fecal strains. In addition, the uroepithelial cells of these animals contain glycosphingolipids and mannoside-containing glycolipids, similar to those of humans, which may act as receptors for various fimbriae (11, 21). Mice infected with *E. coli* or *P. mirabilis* have been shown to develop pyelonephritis (10, 13), and immunization of mice with *E. coli* or *P. mirabilis* fimbriae protects these animals against acute pyelonephritis by homologous and heterologous challenge (14, 27).

Using this model, we observed that 1 week after challenge, the *mrpA* mutant was recovered in significantly smaller numbers in the urine, bladder, and each kidney than was the parent strain. Overall, colonization was reduced by about 6-, 28-, and 18-fold in the urine, bladder, and kidneys, respectively. The histopathologic findings, which showed that the mutant strain generally caused less severe damage to the uroepithelium and did not cause pyelonephritis, further support the bacteriologic results. At the dose of transurethral challenge used in this study, the uropathogenicity of the mutant strain was reduced compared with that of the wild-type strain. These observations indicate that MR/P fimbriae contribute to renal infection by facilitating colonization of the upper urinary tract. The loss of MR/P fimbrial expression, however, did not completely abrogate colonization of the kidneys, indicating that additional virulence factors contribute to the pathogenesis of upper UTI. Nevertheless, the results of this study demonstrate that MR/P fimbriae constitute an important contributing factor in colonization and uropathogenicity of *P. mirabilis*.

Studies in our laboratory with another fimbrial mutant in which the *pmfA* gene (which encodes the major subunit of the

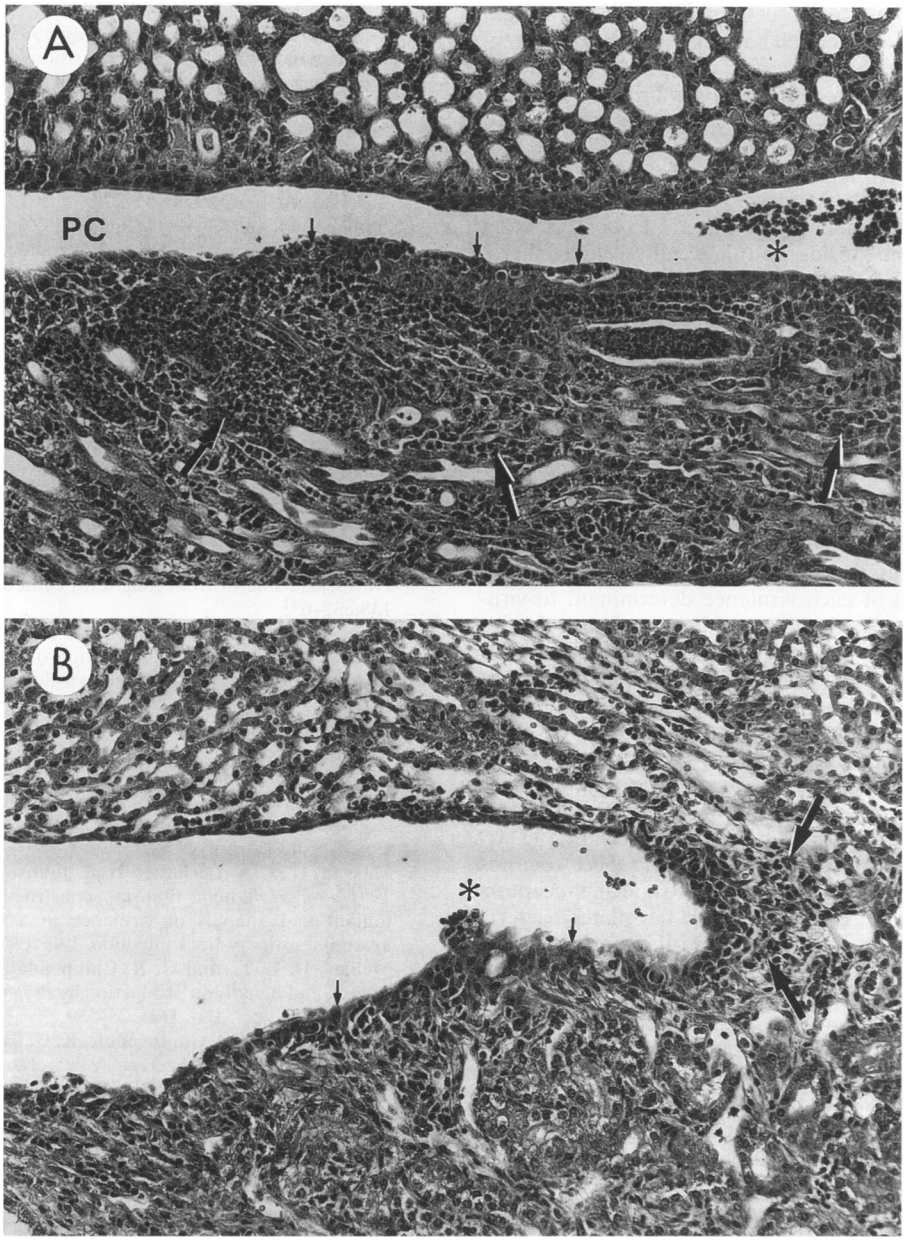

FIG. 7. Representative renal histopathology in mice transurethrally challenged with *P. mirabilis*. (A) Moderate pyelonephritis (renal pathology score, 4+) in the kidney of a mouse challenged with 10^7 CFU of the wild-type *P. mirabilis* HI4320. The inflammatory cell infiltration (large arrows), which is composed of moderate numbers of cells, predominately neutrophils accompanied by mononuclear cells, extends into the parenchyma and is associated with damage to the tubules. Some tubules have been destroyed. Some intact tubules are dilated and contain neutrophilic casts in the lumen. The uroepithelium lining the pelvic cavity is disrupted by neutrophils and local damage (small arrows). The uroepithelium is diffusely thickened. There are moderate numbers of neutrophils (*) in the pelvic cavity (PC). (B) Less severe damage in the kidney of a mouse challenged with the *mrpA* mutant constructed from parent strain HI4320. The *mrpA* mutant caused moderate pyelitis (renal pathology score, 2+) in the kidney of this mouse. The uroepithelial damage is less extensive and milder, resulting in localized thickening of the uroepithelium (small arrows) and focal exfoliation of damaged epithelial cells (*). There are small numbers of inflammatory cells (large arrows) in the parenchyma immediately adjacent to the pelvic cavity.

PMF fimbriae) of strain HI4320 was inactivated within the *P. mirabilis* chromosome demonstrated an 83-fold reduction in colonization of the bladder but was still able to colonize the kidneys in numbers similar to the wild-type strain (16). The observation that mutations in separate fimbrial genes (*mrpA* and *pmfA*) both result in diminished colonization suggests that a number of fimbriae may contribute to colonization of the lower urinary tract but appear to have different consequences on the outcome in the upper urinary tract.

Since there was significantly less colonization by the *mrpA* mutant than by the wild type, it would be logical to assume that a reduction in adherence properties in the kidneys explains this finding. The most obvious target would be receptors on the surface of epithelial cells. However, in vitro adherence to

exfoliated human uroepithelial cells showed no significant difference in the number of bound bacteria between the parent and the mutant. Although we observed no loss of adherence to uroepithelial cells, we know that MR/P fimbriae bind to the surface of human erythrocytes, demonstrating that MR/P fimbriae recognize receptors on human cells. This suggests that the relevant receptor (i) may not have been expressed on the uroepithelial cells, (ii) was not present in a recognizable form on the surface of uroepithelial cells that are to be shed from the urinary tract, or (iii) reside on other cell types or macromolecules present in the urinary tract and may be unique to the renal pelvis. It is also possible that other fimbrial types mediate binding to uroepithelial cells and thus mask, at least partially, the effect of the lack of binding by the *mrp* mutant.

Like other pathogens, *P. mirabilis* has evolved a battery of virulence determinants which appear to act in concert to cause disease. We and others have examined the effect of mutation of other genes on virulence in this model of ascending UTI. Mutants with mutations in urease (13), PMF fimbriae (16), and hemolysin (34) have been separately studied in mouse challenge experiments. We can now include the experiments with the MR/P fimbrial mutant among these studies and rank the apparent contribution of each virulence determinant to virulence as measured by the change in either the 50% infective dose or the number of colonizing organisms between parent and mutant. By using these criteria, urease appears to contribute by far the most significantly to uropathogenicity. This is followed by MR/P fimbriae, which affect colonization levels in the urine, bladder, and kidneys, and then PMF fimbriae, which affect colonization levels only in the bladder. In another study (34), HpmA hemolysin appeared to play no significant role in the development or severity of experimental ascending UTI.

Our current model of virulence of *P. mirabilis* requires MR/P fimbriae and PMF fimbriae to bind to specific receptors on uroepithelium. Once established, motile flagellated bacteria ascend the ureters to the kidney. During this process, urea-induced urease hydrolyzes urea in the urine, causing a rise in local pH which is accompanied by urolithiasis and epithelial cell necrosis. Hemolysin may play some subtle role here but appears to have a more dramatic effect once the organism invades the kidney parenchyma and enters the bloodstream. To validate this model, it will be necessary to construct mutants with additional mutations in flagella and other factors and to use these mutants in challenge studies as described in this report.

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