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Identification and Characterization of the Genes Encoding the Type 3 and Type 1 Fimbrial Adhesins of *Klebsiella pneumoniae*

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Strains of *Klebsiella pneumoniae* are known to express two morphologically and functionally distinct filaments, the type 3 and the type 1 fimbriae. The gene (*mrkD*) encoding the adhesion of *K. pneumoniae* type 3 fimbriae was identified by transcomplementation analysis with the *pap* fimbrial gene cluster of *Escherichia coli*. The nucleotide sequence of the *mrkD* gene was determined. In addition, the determinant coding for the *K. pneumoniae* type 1 fimbrial adhesion was identified, and its nucleotide sequence was deduced. The predicted amino acid sequences of the *K. pneumoniae* adhesion proteins are compared, and similarities with the major fimbrial structural proteins (MrkA and FimA) are discussed.

Type 1 fimbriae of members of the family *Enterobacteriaceae* are filamentous appendages that mediate a mannose-sensitive adherence to eucaryotic cells (23), whereas the morphologically similar Pap pili bind to epithelial cell receptors in the presence of mannose (10). By using the cloned type 1 fimbrial gene cluster and the *pap* gene cluster of *Escherichia coli*, it has been demonstrated that the genes encoding the major fimbrial filament and the receptor-binding specificity are distinct (15, 20). Derivatives of the *pap* genetic system have been constructed such that *E. coli* transformants exhibit fimbriate but nonhemagglutinating (Fim^+ HA^-) as well as nonfimbriate and hemagglutinating (Fim^- HA^+) phenotypes (15). By using complementation analysis with a heterologous gene cluster, the gene encoding the receptor-binding protein (*papG*) has been identified and characterized by nucleotide sequence analysis (17). Also, the *E. coli* type 1 fimbrial gene cluster has been manipulated such that transformants possess a Fim^+ HA^- phenotype (20). Recently, a protein believed to be the terminal tip adhesin of the *E. coli* type 1 fimbriae has been partially characterized (9), and the gene (*fimH*) encoding the adhesion polypeptide has been sequenced (14). In contrast, it has been suggested that the adhesiveness of the K99 fimbriae of *E. coli* is a property of the major fimbrial subunit itself (12), whereas the adhesive protein of the K88 appendages is the major fimbrial protein but is distinct from a minor structural component (11, 22).

Strains of *Klebsiella pneumoniae* commonly express two fimbrial types: the type 1 fimbriae, which are closely related to the type 1 fimbriae of *E. coli*, and the type 3 fimbriae, which are morphologically similar to K88 and K99 fimbriae. Type 1 and type 3 fimbriae are responsible for attachment to fresh guinea pig erythrocytes in the absence of mannose and to tannic acid-treated erythrocytes in both the presence and the absence of mannose, respectively (4). Because all type 1 fimbria-mediated adherence possesses the property of mannose sensitivity, it would be interesting to examine the relatedness of the adhesin polypeptides among different members of the enteric bacteria. In addition, it is unknown whether the other fimbrial types found within the members of the *Enterobacteriaceae* are also composed of fimbrial filament-adhesion complexes, and, if so, to what degree these adhesins differ. In this study, we characterized the gene in *K. pneumoniae* that is analogous to the *fimH* determinant encoding the tip adhesin of E. coli (8). We also present evidence identifying the gene encoding the adhesive property of the type 3 fimbriae.

MATERIALS AND METHODS

Bacterial strains and media. *E. coli* HB101 (18) or ORN103 (24) was used in all transformation experiments and was maintained on Luria agar supplemented with the appropriate antibiotics. All incubations were performed at 37°C for 16 to 20 h.

Hemagglutination assays. For type 1 fimbriae, hemagglutinating activity was determined by using a freshly prepared suspension (3%, vol/vol) of guinea pig erythrocytes. The mannose-resistant, *Klebsiella*-like (MR/K) adhesin of the type 3 fimbriae was detected by using a 3% suspension of tanned human or guinea pig erythrocytes (4). Receptor specificity was determined by the addition of α-methylmannoside for type 1 fimbriae or spermidine for type 3 fimbriae (7a). Hemagglutinating activity and hemagglutination inhibition assays were performed as previously described (2).

Fimbriae were purified as previously described (6). Adherence of cell-free fimbriae to erythrocyte suspensions was detected by a passive hemagglutination assay. One volume of erythrocytes (25 μl) was mixed with one volume of fimbrial protein in the depression of a porcelain tile. Subsequently, 2 μl of fimbrial antiserum was added; after the preparation was mixed for 10 min at ambient temperature, agglutination of erythrocytes was recorded.

Plasmids and plasmid analysis. Construction and characterization of the plasmids encoding the *K. pneumoniae* types 1 (pGG101) and 3 (pFK12) fimbriae, as well as the *E. coli* Pap fimbriae (pDC1), have been described elsewhere (3, 5, 7, 16). The hemagglutination and fimbriation phenotypes of *E. coli* transformants possessing these plasmids and their deletion derivatives are shown in Table 1. Construction of deletion derivatives of pGG101 and pFK12 has been described previously (5, 7). The plasmids used in this study are shown in Fig. 1.

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TABLE 1. Expression of fimbrial type and hemagglutinating activity of *E. coli* transformants

E. coli transformed by:	Surface-associated fimbrial type	Agglutination of erythrocytes from:						
		Human			Guinea pig			
		Untreated[a]	Tanned	Tanned + spermidine[b]	Untreated	Untreated + mannose[c]	Tanned	Tanned + spermidine
pDC1	Pap	+	+	+	−	−	−	−
pDC17	Pap	−	−	−	−	−	−	−
pFK10	Type 3	−	+	−	−	−	+	−
pFK12	Type 3	−	+	−	−	−	+	−
pGG101	Type 1	−	−	−	+	−	+	+
pGG132	Type 1[d]	−	−	−	−	−	−	−

[a] Untreated, 3% suspension of erythrocytes in phosphate-buffered saline; tanned, 3% suspension of erythrocytes treated with tannic acid.
[b] Final concentration of spermidine was 1% (wt/vol).
[c] Final concentration of D-mannose was 1% (wt/vol).
[d] Bacteria possessing this plasmid are agglutinated by type 1 fimbria-specific antiserum but are scantily fimbriate by electron microscopy.

Nucleotide sequencing analysis was performed by the method of Maxam and Gilbert (19). Transcomplementation analyses were performed by using recombinant plasmids possessing the compatible cloning vehicles pBR322 and pACYC184 as previously described (5).

RESULTS

Complementation analysis. The recombinant plasmid pDC17 is a deletion derivative of pDC1 that no longer possesses a functional gene necessary for receptor-binding activity of the Pap pili (*papG*; Fig. 1). Transformants possessing this plasmid produce nonadhesive Pap pili (Fim^+ HA^-) as determined by electron microscopy and reactivity with specific fimbrial antiserum. The phenotypes of *E. coli* possessing pDC17 and derivatives of pFK10 and pFK12 are shown in Table 2. Only in the presence of the *mrkD* gene, carried on pFK52 (Fig. 1), of the type 3 fimbrial gene cluster could pDC17 transformants express the MR/K hemagglutinin. Therefore, *E. coli* containing both pDC17 and pFK52 agglutinated only tanned erythrocytes, and this hemagglutination could be inhibited by spermidine. We have recently shown that this compound specifically inhibits hemagglutinating activity by type 3 fimbriate bacteria, whereas the adhesive activity of type 1 and Pap fimbriae is not affected (7a). Removal of 145 nucleotides from the C-terminal end of the *mrkD* gene (pFK53; Fig. 1) resulted in loss of the ability to complement pDC17 to produce MR/K hemagglutinating activity. Similarly, inversion of the inserted fragment of pFK52 (pFK51; Fig. 1) resulted in loss of hemagglutinating activity by double transformants. Therefore, MR/K hemagglutinating activity was produced only when the *mrkD* gene was located downstream of the promoter of the tetracycline resistance determinant in the cloning vector pACYC184. The *papG* determinant alone did not convert transformants containing an *mrkD* deletion derivative to a hemagglutination phenotype. This is consistent with the hypothesis that the adherence activity of the Pap pili is due to the *papG* determinant plus other ancillary gene products (15–17).

Fimbriae purified from *E. coli* containing pDC17 and pFK52 adhered to tanned erythrocytes in a sperimidine-sensitive manner. Thus, a suspension containing tanned erythrocytes and purified fimbriae isolated from pDC17-pFK52 transformants was readily agglutinated by the addition of specific serum raised against Pap fimbriae. The addition of type 1 or type 3 fimbrial antiserum to the erythrocyte-fimbria suspension did not cause passive hemagglutination. In addition, reaction mixtures containing tanned erythrocytes and the nonhemagglutinating Pap fimbriae, encoded by pDC17, were not agglutinated by the Pap fimbrial antiserum. The fimbriae produced by pDC17-pFK52 transformants were identical in size (approximately 5 nm) to the native Pap fimbriae and were clearly larger than the type 3 appendages, which were between 2 and 3 nm in diameter.

Nucleotide sequence of the *mrkD* gene of *K. pneumoniae*. The nucleotide sequence of the *mrkD* gene is shown in Fig. 2. The predicted molecular mass of the unprocessed gene product was 33,877 daltons, and for the processed polypeptide a molecular mass of 34,000 daltons had been previously reported, using the minicell system (5). No other large open reading frames were found within the appropriate DNA fragment, and the open reading frame was preceded by a potential Shine-Dalgarno consensus sequence. The first 18 amino acids of the polypeptide had the characteristics of a signal peptide, with the cleavage site predicted to be between an alanine and a tryptophan residue (26).

Isolation and characterization of the *K. pneumoniae fimH* gene of the type 1 fimbrial gene cluster. In *E. coli*, the *fimH* gene of the type 1 fimbrial genetic system has been indicated to encode the receptor-specific adhesin (1). The position of the *fimH* gene of *K. pneumoniae* IA565 was determined by minicell analysis of pGG101 and its deletion derivatives.

TABLE 2. Hemagglutinating phenotype of pDC17 *E. coli* transformants containing type 3 fimbrial genes

E. coli (pDC17) transformed by:	*Klebsiella* type 3 fimbrial genes	Agglutination of erythrocytes from[a]:			
		Human			Guinea pig (tanned)
		Untreated	Tanned	Tanned + spermidine	
Untransformed	None	−	−	−	−
pFK20[b]	*mrkA*, *-D*	−	+	−	+
pFK21	*mrkA*, *-B*	−	−	−	−
pFK22	*mrkB*, *-C*, *-D*	−	+	−	+
pFK25	*mrkC*, *-D*	−	+	−	+
pFK52	*mrkD*	−	++	−	++
pFK53	Δ*mrkD*	−	−	−	−

[a] −, No hemagglutination; +, weak hemagglutination after 30 s; ++, strong hemagglutination after 30 s.
[b] The phenotype of *E. coli* transformants possessing only the complementing plasmids is Fim^- MR/K HA^-

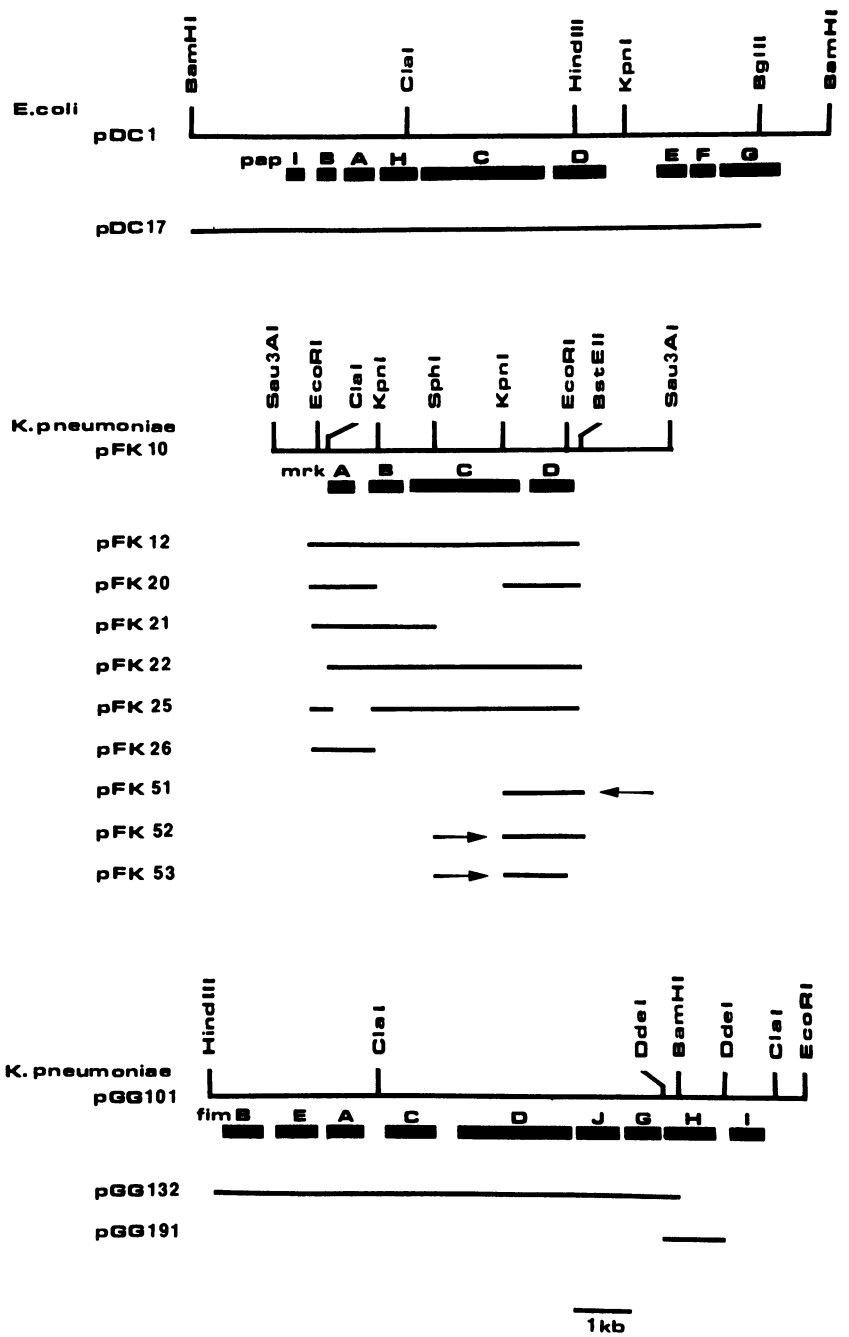

FIG. 1. Physical maps of recombinant plasmids pDC1 (*E. coli* Pap fimbriae), pFK10 (*K. pneumoniae* type 3 fimbriae), and pGG101 (*K. pneumoniae* type 1 fimbriae) and of their deletion derivatives. Locations of the fimbria-associated genes are shown by thick lines. Designations of genes located on pDC1 are according to Lund et al. (16, 17), and the *Klebsiella*-derived genes of pGG101 are similar to those of the *E. coli* type 1 fimbrial system (14). The *K. pneumoniae* type 3 fimbria-associated genes were identified previously (5). The direction of transcription of the *mrkD* gene of pFK51, pFK52, and pFK53 is indicated by arrows. kb, kilobase.

Nucleotide sequencing of this region indicated the presence of one large open reading frame preceded by a Shine-Dalgarno consensus sequence (Fig. 3). The predicted molecular weight of the unprocessed product of the *fimH* gene was calculated to be 31,633. This value is in good agreement with the molecular weight (32,000) for the mature protein, determined by polyacrylamide gel electrophoresis. The N-terminal 23 amino acids had the characteristics of a signal peptide, and the cleavage point was predicted to be between a serine and a phenylalanine residue (26).

A plasmid (pGG191) carrying only the *fimH* gene of *K. pneumoniae* (Fig. 1) could complement, in *trans*, a plasmid (pGG132) carrying most of the remaining genes of the type 1 fimbrial cluster to produce transformants that were fimbriate and hemagglutinating. Such transformants lacked the *fimI* gene (Fig. 1) and were scantily fimbriate, but many intact fimbrial appendages that were not surface associated could be observed under the electron microscope (Fig. 4). Transformants possessing only pGG132 were also scantily fimbriate but nonhemagglutinating, and as with the double

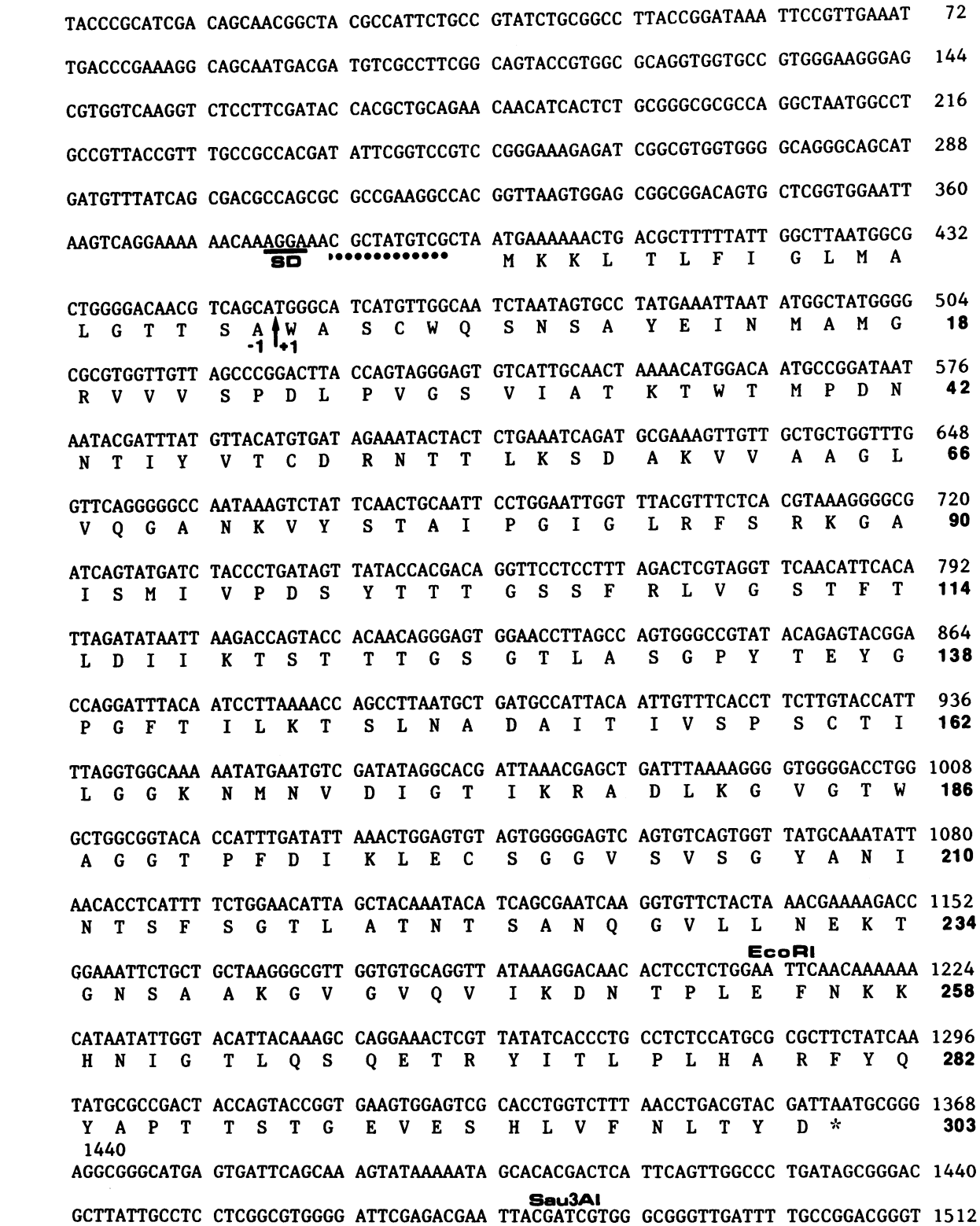

FIG. 2. Nucleotide sequence of the *K. pneumoniae* type 3 fimbrial adhesin gene (*mrkD*) and predicted amino acid sequence of the encoded polypeptide. Nucleotides and amino acids are numbered on the right, the first amino acid of the processed protein being labeled +1. The site of cleavage is marked with an arrow, and the termination codon is indicated with an asterisk. A putative Shine-Dalgarno consensus sequence is labeled SD, and a region with partial dyad symmetry is indicated by the dotted line. The *Sau*3AI site indicates the end of *Klebsiella*-derived DNA in plasmid pFK12 (Fig. 1). The *Eco*RI site of the *mrkD* gene is that shown in Fig. 1.

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                                                    DdeI
CGCCACGCAAGG CACCATTCAGGC GGTGATTAACGT CACCTATACCTA CGCCTGAGCCAG GAGAGCATCATG   72
                                                      SD          M

ATGAAAAAAATA ATCCCCCTGTTC ACCACCCTGCTG CTGCTGGGCTGG TCGATGAACGCC TGGTCCTTTGCC  144
M  K  K  l   I  P  L  F   T  T  L  L   L  L  G  W   S  M  N  A   W  S  F  A    2
      R  V      T         A  V         M  -           V                -1 +1

TGCAAAACGGCC ACCGGGGCGACG ATTCCCATCGGC GGCGGGTCAGCC AACGTCTACGTT AACCTGACCCCG  216
C  K  T  A   T  G  A  T   I  P  I  G   G  G  S  A   N  V  Y  V   N  L  T  P   26
            N     T  A                                                A

GCGGTGAACGTC GGGCAAAACCTG GTGGTCGACCTC TCCACGCAGATT TTTTGCCATAAC GACTATCCGGAA  288
A  V  N  V   G  Q  N  L   V  V  D  L   S  T  Q  I   F  C  H  N   D  Y  P  E   50
V

                                  BamHI
ACGATCACCGAT TACGTGACCCTG CAGCGCGGATCC GCCTATGGCGGT GTGCTGTCGAGT TTTTCAGGCACC  360
T  I  T  D   Y  V  T  L   Q  R  G  S   A  Y  G  G   V  L  S  S   F  S  G  T   74
                                                     N

GTGAAATATAAC GGCACCTCTTAC CCGTTCCCGACC ACCACGGAAACC GCGCGGGTGATT TATGATTCACGG  432
V  K  Y  N   G  T  S  Y   P  F  P  T   T  T  E  T   A  R  V  I   Y  D  S  R   98
         S      S                        S        P        V      N

ACCGATAAACCC TGGCCGGCCGTC CTGTATCTGACG CCGGTGAGCACT GCCGGTGGAGTG GCCATCACCGCA  504
T  D  K  P   W  P  A  V   L  Y  L  T   P  V  S  T   A  G  G  V   A  I  T  A   122
                  V  A                        S                        K

GGATCGTTAATC GCGGTGCTGATC CTGCATCAGACC AACAACTACAAT AGCGACTCCTTC CAGTTCATCTGG  576
G  S  L  I   A  V  L  I   L  H  Q  T   N  N  Y  N   S  D  S  F   Q  F  I  W   146
                              R                       D           V

AACATTTACGCC AACAACGACGTG GTGGTCCCCACC GGCGGCTGCGAC GTCTCCGCCCGC GATGTCACCGTC  648
N  I  Y  A   N  N  D  V   V  V  P  T   G  G  C  D   V  S  A  R   D  V  T  V   170


ACCCTCCCGGAC TACCCGGGATCG ATGGCCGTGCCG CTCACCGTCCAC TGCGCGCAAAGC CAGCAGCTGGGG  720
T  L  P  D   Y  P  G  S   M  A  V  P   L  T  V  H   C  A  Q  S   Q  Q  L  G   194
               R           V  P  I                 Y        K        N

TATTACCTCTCC GGCACCACCGCG GACAGCGCCAAC GCGATCTTCACC AATACCGCCTCC GCCTCGCCGGCG  792
Y  Y  L  S   G  T  T  A   D  S  A  N   A  I  F  T   N  T  A  S   A  S  P  A   218
                  H        A  G      S                          F

CAGGGGATAGGC GTTCAGCTGACG CGCAACGGCAGC GCCGTCCCGGCG AACAGCACGGTC TCGCTGGGCACC  864
Q  G  I  G   V  Q  L  T   R  N  G  S   A  V  P  A   N  S  T  V   S  L  G  T   242
      V                             T   I  I           N                 A

GTCGGCACCTCG CCGGTCAACCTC GGCCTGACGGCC ACCTATGCCCGG ACCACAGGCCAG GTTACCGCCGGC  936
V  G  T  S   P  V  N  L   G  L  T  A   T  Y  A  R   T  T  G  Q   V  T  A  G   266
             A     S           N                       G

AACGTGCAGTCG ATCATCGGCATC ACCTTTGTCTAT CAATGATGACCG ATTATATCCTCT CGCCCTGCTCGC 1008
N  V  Q  S   I  I  G  I   T  F  V  Y   Q  *                                   279
                  V

TGGCCGCCCGCG GGTTGAGCCAGC TGATGCTGAA                                        1042
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FIG. 3. Nucleotide sequence of the *K. pneumoniae fimH* gene and predicted amino acid sequence of the encoded gene product. The predicted site of cleavage of the processed polypeptide is shown by the arrow, and the termination codon is indicated with an asterisk. The Shine-Dalgarno consensus sequence is labeled SD. The *Dde*I site upstream of the *fimH* gene is that shown to the left of the *Bam*HI site in Fig. 1. Positions at which the predicted amino acid sequence of the *fimH* gene of *E. coli* (14) differ from that of *K. pneumoniae* are indicated below the *Klebsiella* sequence. The absence of a leucine residue in the signal peptide of the *E. coli fimH* gene product is indicated by a dash.

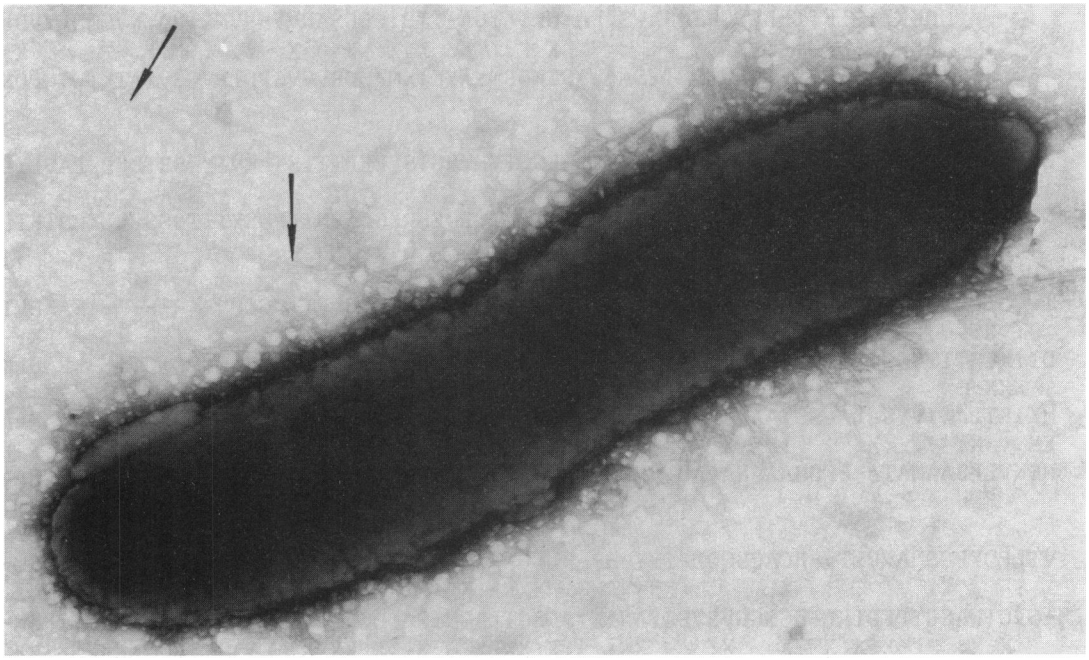

FIG. 4. Electron micrograph of *E. coli* HB101 containing pGG132 and pGG191. Type 1 fimbriae, both cell associated and unattached, are indicated by arrows.

transformant described above, many fimbrial filaments were detached from the bacterial surface. Transformation of *E. coli* carrying pDC17 with pGG191 did not result in a hemagglutination phenotype.

Comparison of the MrkA, FimA, MrkD, and FimH proteins. The amino acid sequences of the *K. pneumoniae* type 1 and type 3 major fimbrial subunit proteins (*fimA* and *mrkA* gene products) as well as the putative adhesins (*fimH* and *mrkD* gene products are shown in Fig. 5. The 15 C-terminal residues of all four proteins demonstrated 40 to 60% sequence similarity. The processed FimH and MrkD proteins had 40% amino acid similarity within the first 15 N-terminal residues, and the processed FimA and MrkA proteins shared 7 of the first 14 amino acids (50% identity). However, the N-terminal sequences of the fimbrial structural proteins (FimA and MrkA) did not resemble those of the putative adhesins (FimH and MrkD). Overall, the positions of approximately 29% of specific amino acid residues within the FimA and MrkA proteins were conserved. In the case of the adhesins (FimH and MrkD), 18% of the residues were in similar positions. The amino acid sequence of the PapG polypeptide (17) exhibited little similarity to those of the FimH, FimA, MrkA, and MrkD proteins.

DISCUSSION

Recent observations have indicated that two types of *E. coli* fimbriae (type 1 and Pap), unlike the K99 fimbriae, are composed of a structural fimbrial filament and a distinct adhesin (1, 8, 12, 13, 17, 22). The *fimH* gene product of the type 1 fimbrial system has been indicated to be the tip adhesin of appendages demonstrating mannose-sensitive receptor-binding specificity (9); for the Pap fimbriae, the *papG* gene product appears to be the adhesive moiety (17). Most strains of *K. pneumoniae* also produce type 1 fimbriae as well as type 3 fimbriae mediating MR/K adherence. We present evidence that, as with the *E. coli* type 1 and Pap fimbriae, the adhesive properties of the *Klebsiella* fimbriae are due to the presence of gene products separate from the major fimbrial filament.

Phenotypic expression of functional type 3 fimbriae is due to the expression of at least four genes (*mrkA*, *-B*, *-C*, and *-D*; 5). Because previous results had indicated that deletions within the *mrkD* gene resulted in transformants that possessed type 3 fimbriae but no longer agglutinated tanned erythrocytes (5), a plasmid (pFK52) carrying only the *mrkD* gene was constructed. When this plasmid was introduced into a transformant able to express nonadhesive Pap fimbriae, the double transformants were able to express fimbriae that were antigenically and morphologically those of the Pap type but mediated agglutination only of tanned erythrocytes. Thus, pDC17 possesses all of the genes necessary for expression of Pap fimbriae (F11 serotype; 3) but lacks the gene encoding erythrocyte-binding activity. *E. coli* transformants containing pDC17 are correspondingly fimbriate but nonhemagglutinating (Fim^+ HA^-). Acquisition of plasmid pFK52 by pDC17 transformants resulted in fimbriae that were serologically Pap but of the MR/K type in hemagglutinating activity. These results strongly suggest that the *mrkD* gene product is responsible for conferring the MR/K hemagglutinating activity of *Klebsiella* type 3 fimbriae. The complete gene cluster (pDC1) of the Pap fimbriae encodes appendages that mediate agglutination of fresh erythrocytes of only human origin, both in the presence and in the absence of spermidine. It is unlikely that the *mrkD* gene indirectly converts the Pap fimbriae to recognize only tanned erythrocytes of both human and guinea pig origin and to exhibit sperimidine sensitivity unless the *mrkD* gene product itself is the type 3 fimbrial adhesin.

The nucleotide sequence analysis of a DNA fragment containing the proposed *mrkD* gene revealed a single open reading frame, and the size of the predicted gene product

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FimH        MMKKIIPLFTTLLLLGWSMNAWS↑FA-CKTATGA-TIPIGGGSANVY--VNLTPAVNVGQNLVVDL
             XXX:       : X     : :  X X  :  X  X X  X   X   : :   :      : X
MrkD         MKKLT----LFIGLMALGTTSA↑WASCWQSNSAYEINMAMGRVVVSPDLPVGSVIATKTWTMPDN

FimH  ST-QIFC-HNDYPETITDYVTLQRGSAYGGVLSSFSGTVKYNGTSYPFPTTTETARVIYDSRTDKPWPAVLYL
       X  : X  X    : :  X:          X X:    :    :      :              : :   :   X
MrkD  NTIYVTCDRNTTLKSDAKVVAAGLVQGANKVYSTAIPGIGLRFSRKGAISMIVPDSYTTTGSSFRLVGSTFTL

FimH  TPVSTAGGVAI-TAGSLIAVLIL HQTNNYNSDSFQF-IWNIYANNDVVVPT-GGCDV---------SARDVT
        : X:       X  X                :       X  :      ::    XX  :            X:
MrkD  DIIKTSTTTGSGTLASGPYTEYG PGFTILKTSLNADAITIVSPSCTIL----GGKNMNVDIGTI--KRADLK
        XXX :      :XX    :        X:   ::       :X  :X::     X     X :X :  : X X
FimA  MKIKTLAMIVVSALALSSTAALA↑DTTTVNGGTVHFKG-EVVNAACAVDA---GSIDQTVQLGQV--RSAKLA
       XX   X:  : :X     :XX X   X X XX X X X  X   :X:X     XX X  X X  X     X X
MrkA  MKKVLLSAAMATA-FFGMAAANA↑ADTNVGGGQVNFFG-KVTDVSCTVSVNGQGS-DANVYLSPVTLTEVKAA

FimH  VTLPDYPGSMAVPLTV-HCAQSQQL-GYY-----LSG--TTADSANAI--FTN-TASASPAQGIGVQLTRNG-
        :    X     : :  X:    : XX       XX  :X  XXX      X  :  X X X:XXX: :
MrkD  -GVGTWAGGTPFDIKL-ECSGGVSVSGYANINTSFSGTLATNTSANQGVL-LNEKTGNSAAKGVGVQVIKDN-
         X: :    X X X :X   X:    X :  :X:XX X  :X  X XX X:  :   :X  XXXX:: XX
FimA  -TAGSTSSAVGFNIQLDDCDTTVATK--ASV--AFAGT-AIDSSNTT-VLALQNSA-AGSATNVGVQIL-DNT
       :X :      X X : XX ::  XX    X  :  X   :  :    :XX   :   X  :X         X
MrkA  -AADTYLKPKSFTIDVSDCQAADGTK-QDDV--SKLG---VNWTGGN-LLAGATAKQQGYLANTEAAGA-QNI

FimH  -SAVPAN--STVSLGTV---GTSPV-------NLGLTAT------YARTT-GQVTAGNVQSIIGITFVYQ
       : :  X      :XX:    X  :        X X X       XX       ::X X X :     X
MrkD  -TPLEFN--KKHNIGTLQSQETRYI-------TLPLHARF---YQYA----PTTSTGEVESHLVFNLTYD
       XXX  X        XX:  :   X        :X  XX      XX     ::::X   :   X : X:
FimA  GTPLALNGATFSAATTLN--DDPNI--------IPFQAR-----YYAT---GAATAGIANADATFKVQYE
         X:   XX : :  :   X  :          :   XX      XXX    : X:X: X: XX: : X
MrkA  QLVLSTDNAT-ALTNKIIPGDSTQPKAAGDASAVQDGARFTYYVGYATSTPTTVTTGVVNSYATYEITYQ
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FIG. 5. Relatedness of fimbria-associated proteins. Amino acids are given by standard one-letter codes. X, Amino acid identity; :, isofunctional residues (K and R; A, S, and T; Y and F; Q and N; D and E; I, L, M, and V). The processing sites of cleavage of the signal peptides are marked with arrows.

was in good agreement with that shown by minicell analysis (5). Two possible initiation codons are located downstream of the putative Shine-Dalgarno sequence, at positions 388 and 396 (Fig. 2). The second codon precedes two lysine codons and is therefore likely to represent the translation initiation site of the *mrkD* gene. The distance (15 nucleotides) between this site and the Shine-Dalgarno consensus sequence could be decreased by formation of a hairpin loop structure resulting from a small palindromic sequence in this region. The formation of a similar structure has been hypothesized for gene 38 of *E. coli* bacteriophage T4 (25). Alternatively, the initiation codon at position 388 (Fig. 2) may encode the first amino acid residue of the *mrkD* gene product. However, in this case the N-terminal region of the signal peptide would not correspond to those of other known signal peptides.

It was interesting to note that transformants containing both pDC17 and pFK51 did not demonstrate MR/K hemagglutinating activity. The only difference between pFK51 and pFK52 is the orientation of the *Klebsiella*-derived DNA fragment within the cloning vehicle (Fig. 1). When the *mrkD* gene was placed in the correct orientation downstream of the promoter of the tetracycline resistance determinant, the double transformants were MR/K HA^+. In the opposite orientation, the transformants were MR/K HA^-, which suggested that the *mrkD* gene is expressed from the relatively strong promoter of the cloning vector (pACYC184). We have recently reported that another *Klebsiella* gene (*mrkA*) of the type 3 fimbrial cluster is expressed more strongly in *E. coli* when it appears to be expressed from a heterologous promoter (5). Therefore, the lack of MR/K hemagglutinating activity by pFK51 transformants is probably due to poor expression of this gene in the *E. coli* host.

Recent reports by our group have shown that the type 1 fimbrial gene cluster of *K. pneumoniae*, like that in *E. coli*, consists of several genes (7). The *fimH* gene of *K. pneumoniae* is located in a position similar to that of the analogous gene of *E. coli* and encodes a polypeptide of approximately the same size. Recently, Hanson and Brinton (8) and Abraham et al. (1) have suggested that the *E. coli* FimH protein is the adhesin of type 1 fimbriae. Nucleotide sequencing analysis of the *E. coli fimH* gene has been reported by Klemm and Christiansen (14). Nucleotide sequencing analysis of the *Klebsiella fimH* gene demonstrates that the gene product is

very similar but not identical to that of *E. coli*. The FimH polypeptides of *E. coli* and *K. pneumoniae* demonstrate an amino acid sequence agreement of 85%. This value is similar to the relatedness of the FimA proteins of the two systems, in which the amino acid sequence agreement is 80% (7, 13).

Complementation of a Fim^+ HA^- recombinant by plasmids carrying the *fimH* gene did result in the expression of *Klebsiella* type 1 fimbriae. However, we have not been able to determine that the *fimH* gene product is solely responsible for the binding activity because, unlike the *mrkD* gene, pGG191 does not convert *E. coli* transformants containing plasmids encoding nonadhesive Pap fimbriae to the mannose-sensitive receptor-binding phenotype. Therefore, it is possible that the FimH protein is a major component of the type 1 adhesin but requires additional fimbrial polypeptides to be fully functional. Alternatively, the FimH of *K. pneumoniae* may be the sole adhesin protein but may not be integrated into the Pap fimbriae because of an inability to associate with the structural proteins of this fimbrial system. Our current results do not differentiate between these two possibilities.

The conserved nature of the C termini of various fimbria-associated proteins has been reported (21). Our results also indicate that both adhesin and structural fimbria-associated proteins demonstrate significant sequence agreement between each other, particularly in the C-terminal region. It is possible that the genes encoding the fimbrial polypeptides of members of the *Enterobacteriaceae* have evolved from a single determinant. Therefore, the K99 system, in which adhesive activity is associated with the fimbrial subunit (12), represents one of the most rudimentary fimbrial complexes. However, the type 1 and type 3 fimbrial clusters have evolved to carry the genes encoding adhesiveness and fimbrial filament formation on two independent loci. Jacobs and co-workers (11) have suggested that the major component of the K88 fimbriae is the adhesin molecule. In the case of type 1 and type 3 fimbriae, the adhesin appears to be a minor component of the appendages, and therefore enteric fimbrial gene clusters (Pap, type 1, type 3, K88, K99, etc.) have evolved such that each system produces specific relative amounts of fimbrial components but retains a fundamental genetic organization. However, because of the lack of DNA sequence agreement between genes from distinct fimbrial gene clusters, the possibility of convergent evolution among these fimbrial determinants cannot be ruled out.

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