matB, a Common Fimbrillin Gene of Escherichia coli, Expressed in a Genetically Conserved, Virulent Clonal Group

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A novel fimbrial type in Escherichia coli was identified and characterized. The expression of the fimbria was associated with the O18acK1H7 clonal group of E. coli, which cause newborn meningitis and septicemia when grown at low temperature; hence, it was named the Mat (meningitis associated and temperature regulated) fimbria. The fimbriae were purified from a fimA::cat sfaA::Gm fliC::St derivative of the O18K1H7 isolate E. coli IHE 3034. The purified Mat fimbrillin had an apparent molecular mass of 18 kDa and did not serologically cross-react with the type 1 or S fimbria of the same strain. The matB gene encoding the major fimbrillin was cloned from the genomic DNA of the fimA::cat sfaA::Gm fliC::St derivative of IHE 3034. The predicted MatB sequence was of 195 amino acids, contained a signal sequence of 22 residues, and did not show significant homology to any of the previously characterized fimbrial proteins. The DNA sequence of matB was 97.8% identical to a region from nucleotides 17882 to 18469 in the 6- to 8-min region of the E. coli K-12 chromosome, reported to encode a hypothetical protein. The 7-kb DNA fragment containing matB of IHE 3034 was found by restriction mapping and partial DNA sequencing to be highly similar to the corresponding region in the K-12 chromosome. Trans complementation of the matB::cat mutation in the IHE 3034 chromosome showed that matB in combination with matA or matC restored surface expression of the Mat fimbria. A total of 27 isolates representing K-12 strains and the major pathogroups of E. coli were analyzed for the presence of a matB homolog as well as for expression of the Mat fimbria. A conserved matB homolog was found in 25 isolates; however, expression of the Mat fimbriae was detected only in the O18acK1H7 isolates. Expression of the Mat fimbria was temperature regulated, with no or a very small amount of fimbriae or intracellular MatB fimbrillin being detected in cells cultivated at 37°C. Reverse transcriptase PCR and complementation assays with mat genes controlled by the inducible trc promoter indicated that regulation of Mat fimbria expression involved both transcriptional and posttranscriptional events.

Numerous proteinaceous adhesins have been detected in Escherichia coli (for recent reviews, see references 20 and 27). These adhesins occur in the form of fimbrial filaments or are nonfimbrial proteins of the outer membrane. The adhesins recognize different receptor molecules on the mammalian epithelia or extracellular matrices and function to enable colonization of E. coli at specific ecological niches. Many of the adhesins are associated with E. coli isolates from specific disease manifestations and contribute to the establishment of the infections. Examples of such disease-associated adhesins include the P fimbria of uropathogenic E. coli (UPEC) (55) and the various adhesin types detected in E. coli pathogroups causing diarrheal diseases (reviewed in references 13 and 36). Some adhesin genes, such as those encoding the mannoside-binding type 1 fimbriae (26) and the fibronectin-binding curli (39), are present on and expressed by a large proportion of natural E. coli isolates. The type 1 fimbriae are important for the spread of E. coli from one host individual to another (6) and also enhance bacterial colonization in the human and animal gut and the urinary tract (20, 27). The curli are expressed at low temperature and low osmolarity and may convey

a selective advantage for E. coli in the environment and in the early phases of intestinal tract colonization (40).

The natural populations of E. coli exhibit extensive genetic diversity that is organized into a limited number of genetically distinct clonal groups (reviewed in reference 59). Such widespread, homogenous clonal groups have been well characterized in E. coli strains from separate outbreaks of disease. The isolates in the clonal groups are characterized by several identical phenotypic traits, such as serotype, biotype, phage type, outer membrane protein profiles, and production of hemolysins, other toxins, specific iron-scavenging systems, and specific adhesins. Multilocus enzyme electrophoresis has shown that isolates in a given clonal group are genetically conserved (50, 59), and the clonal groups appear sufficiently stable that they have spread into human populations on several continents. Clonal groups in pathogenic E. coli include several UPEC clones (10, 43, 55), enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC), and enteroaggregative E. coli (EAEC) clones (reviewed in references 13 and 35). The association of specific adhesins with these pathogroups has resulted from horizontal gene transfer of plasmid genes or chromosomal pathogenicity islands from other pathogens. On the other hand, diversification of adhesin alleles may result from within-host evolution of E. coli, in which random point mutations affect the tissue tropism of the bacterium (52).

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The O18acK1H7 isolates represent the major clonal group of *E. coli* associated with newborn meningitis and septicemia (MENEC) (1, 31, 51). Electrophoretic analysis of chromosomally encoded enzymes revealed that the O18acK1H7 MENEC isolates are genetically highly conserved and form a distinct clone, which is phenotypically characterized by expression of the S and the type 1 fimbriae and a conserved outer membrane protein profile, as well as, on the other hand, by lack of the P and the type 1C fimbriae and hemolysin (1, 31, 51). In this report, we describe a novel fimbrial gene that is common in *E. coli* isolates, including laboratory K-12 strains, but is expressed only in O18acK1H7 MENEC.

MATERIALS AND METHODS

Bacteria, growth conditions, and plasmids. The bacterial strains and plasmids used in this work are listed in Table 1. For expression studies, the bacteria were cultivated in static Luria broth at 20 or 37°C and under shaking at 37°C for other purposes. Antibiotics, when necessary, were added at concentrations of 100 (ampicillin), 25 (chloramphenicol), 30 (gentamicin [Gm]), 75 (rifampin [Rif]), 100 (streptomycin [Sm] and streptothricin [St]), or 12.5 (tetracycline) μg ml^{-1}. The antibiotics were obtained from Sigma Chemical Co, St Louis, Mo., except for streptomycin and streptothricin, which were a gift from Helmuth Tschäpe, Robert Koch Institute, Wernigerode, Germany. Induction of *mat* genes in recombinant *E. coli* strains was done with 5 μM isopropyl-β-D-thiogalactopyranoside (IPTG) (Promega Corporation, Madison, Wis.).

The influence of growth conditions on expression of Mat fimbria was tested by cultivating the bacteria in static Luria broth at 20 or 37°C, as well as at 37°C in a 5% (vol/vol) CO_2 atmosphere, under anaerobic conditions, in the presence of 0.2% (wt/vol) D-glucose, with a low (0.1%) or high (2%) concentration of NaCl, in the presence of 0.2 mM iron chelator 2,2-dipyridyl (Sigma Chemical Co.), or in the presence of 10% (vol/vol) human whole blood. The bacteria were passaged three times under these conditions before they were tested for Mat fimbria expression.

DNA techniques. Isolation of plasmid and chromosomal DNA from *E. coli* cells and DNA manipulations were performed by routine procedures (47). Restriction enzymes (Promega Corp., Madison, Wis., and New England Biolabs, Beverly, Mass.) were used according to the manufacturers' instructions. Cloning of IHE 3034–79 chromosomal DNA into cosmid pHC79 was done according to the manufacturer's instructions (DNA-Packaging kit; Boehringer Mannheim, Mannheim, Germany), resulting in the plasmid pMAT1. A 7-kb *Eco*RI fragment of pMAT1 that reacted in Southern hybridization (47) with the degenerate oligonucleotides designed on the basis of the peptide sequences obtained from MatB was subcloned into pACYC184 to give pMAT2. The DNA sequences of the degenerate oligonucleotides used in the hybridization were 5′ GCIGAIGT IACIGCICAIGCIGTIGCIACITGG 3′ and 5′ TTIGAIGTIGCIATIGAIGG IGAI 3′. The nucleotide sequence of the DNA region encoding *matA* and *matB* in pMAT2 was determined by using a commercially available sequencing service (MedProbe AS, Oslo, Norway). The *matB* gene in the chromosome of clinical *E. coli* isolates was detected by PCR with primers designed on the basis of the sequence of the *matB* homolog of MG1655 (5) and DyNAzyme II DNA-polymerase (Finnzymes, Espoo, Finland) according to manufacturer's instructions. The obtained PCR products were analyzed by Southern hybridization with *matB* gene of *E. coli* IHE 3034 as a probe by using the ECL enhanced chemiluminescence direct nucleic acid labeling and detection system (Amersham Pharmacia Biotech, Amersham Place, Little Chalfont, Buckinghamshire, England) according to the manufacturer's instructions.

For expression, DNA fragments representing different regions of the *mat* gene cluster were amplified by PCR with *Pfu* polymerase (Stratagene, La Jolla, Calif.), pMAT2 as a template, and primers designed on the basis of the DNA sequence of the *mat* region in *E. coli* MG1655 (5). The forward primer contained an *Eco*RI restriction site, and the reverse primer contained a *Sal*I restriction site at the 5′ end. The amplified DNA fragments were cloned into the *Eco*RI-*Sal*I-site of pSE380 and used in the complementation assays.

For reverse transcriptase PCR (RT-PCR), total RNA from *E. coli* strains IHE 3034, IHE 3034–90, and MG1665 was extracted with Qiagen Rneasy kit, following the manufacturer's guidelines. The enhanced avian RT-PCR kit (Sigma) and a PTC-200 Peltier thermal cycler (MJ Research) were used to amplify mRNA by the two-step method according to the manufacturer's instructions. The same amounts of template RNA were used for comparing the *mat* transcripts; the amounts varied from 0.5 to 2.0 μg in different experiments. Specific primers

corresponded to the following nucleotides of the *E. coli* MG1665 sequence available in GenBank (accession no. U73857): *matA* forward, 19117 to 19134; *matA* reverse, 18547 to 18569; *matB* forward, 18449 to 18469; *matB* reverse, 17882 to 18002; *matC* forward, 17807 to 17824; *matC* reverse, 17156 to 17176. Amplimers were separated by electrophoresis in a 1% agarose gel and stained with ethidium bromide; their relative amounts were evaluated from the gels by using the Tina (version 2.0) image analysis program (Raytest Isotopenmessgeräte Gmbh, Straubenhardt, Germany).

A *fimA sfaA fliC* triple mutant of IHE 3034, named IHE 3034–79, was constructed by inserting the *sat* gene encoding streptothricin acetyltransferase from pIE934 (54) into the *Nco*I site within the *cat* gene in a *fliC::cat* fusion (58) cloned to the suicide plasmid pGP704 (34). The resulting plasmid was used to silence the *fliC* gene of the *fimA::cat sfaA::*Gm derivative IHE 3034–59 (45) by allelic replacement essentially as described in reference 58. A MatB-null variant of IHE3034, named *E. coli* IHE 3034–90, was constructed by inserting a *cat* cassette encoding chloramphenicol acetyltransferase from pMMS1 (58) into the *Bsu*36I site in *matB* by the same methodology described above. The correct genotypes of IHE 3034–79 and IHE 3034–90 were confirmed by Southern hybridization with *fliC* and *sat* as DNA probes for IHE 3034–79 and *matB* and *cat* for IHE 3034–90. The correct phenotypes of IHE 3034–79 and IHE 3034–90 were confirmed by agglutination in an antiserum against H7 flagella available from previous work (58) or in an antiserum against the Mat-fimbriae obtained in this study as well as by immunoelectron microscopy (IEM).

Sequence comparisons. Homology searches in the databases were performed with the BLAST (available at http://www.expasy.ch/sprot/sprot-top.html) and FASTA (http://www.ebi.ac.uk/fasta3/) package programs. Comparison of MatB with other fimbrillins was done by using Align for global alignment (http://molbiol.soton.ac.uk/compute/align.html) and SIM for local alignment (http://www.expasy.ch/tools/sim-prot.html). Prediction of the presence of stem-loop structures and signal sequences in the *mat* genes of MG1655 and IHE 3034 was done by using programs available at http://www.fruitfly.org/seq_tools/promoter.html, http://www.genebee.msu.su/services/rna2_reduced.html, and http://www.cbs.dtu.dk/services/SignalP/. DNA-binding motifs were identified by using the ProfileScan program at http://www.isrec.isb-sib.ch/.

Protein chemical methods. Mat fimbriae were purified from *E. coli* IHE 3034–79 and *E. coli* LE392(pMAT1) by using deoxycholate and concentrated urea (29). Prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (47), the type 1 and Mat fimbriae were denatured at low pH (33).

Protein and peptide sequencing was performed with an Applied Biosystems 494A Procise sequencer. For analysis the protein was, after SDS-PAGE, electroblotted onto a ProBlott (Applied Biosystems, Perkin-Elmer, Foster City, Calif.) membrane. For internal sequencing, the protein was digested with trypsin, the peptides generated were separated by reverse-phase chromatography, and a selected peptide was subjected to analysis (61).

Immunological methods. The anti-type 1 fimbria and anti-Mat fimbria sera were obtained by immunizing rabbits according to standard procedures with the type 1 fimbriae purified from *E. coli* IHE 3034–8 and the Mat fimbriae purified from *E. coli* IHE 3034–79 and *E. coli* LE392(pMAT1) used as antigens. The rabbit antiserum raised against the S fimbriae of IHE 3034 was available from previous work (56). Polyclonal anti-Mat, anti-type 1, and anti-S fimbria sera were titrated against the purified type 1, S, and Mat fimbriae by enzyme-linked immunosorbent assay (ELISA) as described previously (30) with alkaline phosphatase-conjugated swine anti-rabbit immunoglobulins (Dako A/S, Denmark; diluted to 1:500) as secondary antibodies and phosphatase substrate (Sigma Diagnostics, Inc., St. Louis, Mo.) at a concentration of 1 mg/ml in diethanolamine-magnesium chloride buffer (OY Reagena AB, Kuopio, Finland). Results were recorded with a Multiscan MS ELISA reader (Labsystems OY, Helsinki, Finland). Western blotting of SDS-PAGE-separated fimbrial proteins with anti-Mat fimbria antibodies was done by routine procedures as described in reference 11.

Mat fimbria expression on *E. coli* strains was detected by indirect immunofluorescence according to the procedure described in reference 42. Briefly, the bacteria were immobilized on glass slides, fixed with 3.5% (wt/vol) paraformaldehyde in phosphate-buffered saline (PBS), and stained with anti-Mat fimbria antibodies diluted 1:50 and with fluorescein isothiocyanate (FITC)-labeled swine anti-rabbit immunoglobulins (Dako A/S) diluted 1:50. Stained bacteria were mounted in nicetamid and examined in an Olympus BX50 fluorescence microscope (Olympus Optical Co., Hamburg, Germany) equipped with epi-illumination and interference filters for FITC. For detection of Mat fimbria expression by whole-cell ELISA as described in reference 8, the bacteria were immobilized onto ELISA plates (Nunc A/S, Roskilde, Denmark) for 16 h at 37°C, washed, and left to react with anti-Mat antibodies diluted from 10^{-2} to 10^{-7}, and the amount of bound antibodies was measured by ELISA as described above.

TABLE 1. *E. coli* strains and plasmids used in this study

Strain	Relevant characteristics	Source or reference	Presence of *matB*[a]	Expression of Mat fimbriae[b]
Strains				
IHE 3034	018:K1:H7, MENEC	31, 51	+	+
IHE 3035	018:K1:H7, MENEC	31, 51	+	+
IHE 3040	018:K1:H7, MENEC	31, 51	+	+
IHE 3047	018:K1:H7, MENEC	31, 51	+	+
IHE 3080	018:K1:H7, MENEC	31, 51	+	+
IHE 1041	01:K1:H7, UPEC	55	+	−
IHE 1049	01:K1:H7, UPEC	55	+	−
IHE 1086	04:K12:H7, UPEC	55	+	−
IHE 1402	06:K2:H1, UPEC	55	+	−
IHE 1431	06:K2:H1, UPEC	55	+	−
IHE 1190	018:K5:H7, UPEC	55	+	−
IHE 1268	018:K5:H7, UPEC	55	+	−
IHE 1106	06:K13:H1, UPEC	55	+	−
IHE 1152	06:K13:H1, UPEC	55	+	−
536	06:K15:H31 UPEC	16	+	−
RHE 4260	EAEC	23, 24	+	−
IHE 10162	EAEC	KTL, Helsinki, Finland	+	−
ATCC 43895	0157:H7, EHEC		+	−
IHE 53430	0157:H7, EHEC	41	+	−
RHE 4283	EPEC	KTL, Helsinki, Finland	+	−
IHE 110156	EPEC	KTL, Helsinki, Finland	+	−
RHE 3532	ETEC	KTL, Helsinki, Finland	−	−
IHE 96057	ETEC	23, 24	−	−
LE392	K-12	Promega	+	−
MG1655	K-12	5	+	−
AAEC072A	K-12	7	+	−
XL1 Blue MRF′	K-12	Stratagene	+	−
IHE 3034-Sm	Streptomycin-resistant IHE 3034	45		
IHE 3034-2	IHE3034 *fimA::cat*	45		
IHE 3034-8	IHE3034 *sfaA*::Gm	45		
IHE 3034-59	IHE3034 *fimA*::cat *sfaA*::Gm	45		
IHE 3034-79	IHE3034 *fimA*::cat *sfaA*::Gm *fliC*::sat	This study		
IHE 3034-Rif	Rifampin-resistant IHE 3034	This study		
IHE 3034-90	IHE 3034 *matB*::cat	This study		
Plasmids				
pHC79	Cosmid vector	19		
pACYC184	Plasmid vector	New England Biolabs		
pSE380	Expression vector, *trc* promoter	Invitrogen		
pMAT1	IHE3034 *mat* genes in ~40 kb *Sau*3 AI fragment in pHC79	This study		
pMAT2	3034-79 *matABC orf1, -2,* and *-3* in 7 kb *Eco*RI fragment in pACYC184	This study		
pMAT3	*matABC orf1, -2, and -3* in pSE380	This study		
pMAT4	*matABC* in pSE380	This study		
pMAT5	*matAB* in pSE380	This study		
pMAT6	*matBC orf1, -2,* and *-3* in pSE380	This study		
pMAT7	*matBC orf1, and -2* in pSE380	This study		
pMAT8	*matBC orf1* in pSE380	This study		
pMAT9	*matBC* in pSE380	This study		
pMAT10	*matB* in pSE380	This study		
pMAT11	*matC orf1, -2,* and *-3* in pSE380	This study		
pMMS1	*fimA*::cat in pBluescript II KS+	58		
pIE934	*sat*	54		
pMRK1	*pla* of *Yersinia pestis* in pSE380	M. Kukkonen		

[a] Detected by PCR and DNA hybridization with IHE 3034 *matB* as a probe.
[b] Detected in cells cultivated at 20°C by indirect immunofluorescence microscopy and agglutination with anti-Mat fimbria antiserum.

Electron microscopy. For electron microscopy, the bacteria were suspended in Luria broth and immobilized on copper grids coated with Pioloform and carbon. For negative staining, the bacteria were stained with 1% (wt/vol) potassium tungstate (pH 6.5) for 1 min. For IEM, the bacteria were left to react with anti-Mat antibodies diluted to 1:500 in PBS and 10-nm-particle-diameter gold-labeled protein A (Amersham Life Science) diluted to 1:40 and negatively stained as described above. The grids were examined in a JEOL JEM-1200EX transmission electron microscope at an operation voltage of 60 kV. The micro-graphs were taken at the Section of Electron Microscopy, Institute of Biotechnology, University of Helsinki.

Hemagglutination assays. Treatment of human O₁ erythrocytes with endo-β-galactosidase, neuraminidase, trypsin, and pronase and agglutination of the erythrocytes by *E. coli* 3034–79 were performed by routine procedures (28).

Nucleotide sequence accession number. The nucleotide sequence of the 6- to 8-min region of the *E. coli* MG1655 chromosome (5) is available at GenBank under accession no. U73857, and the sequence of the *matAB* region of the *E. coli*
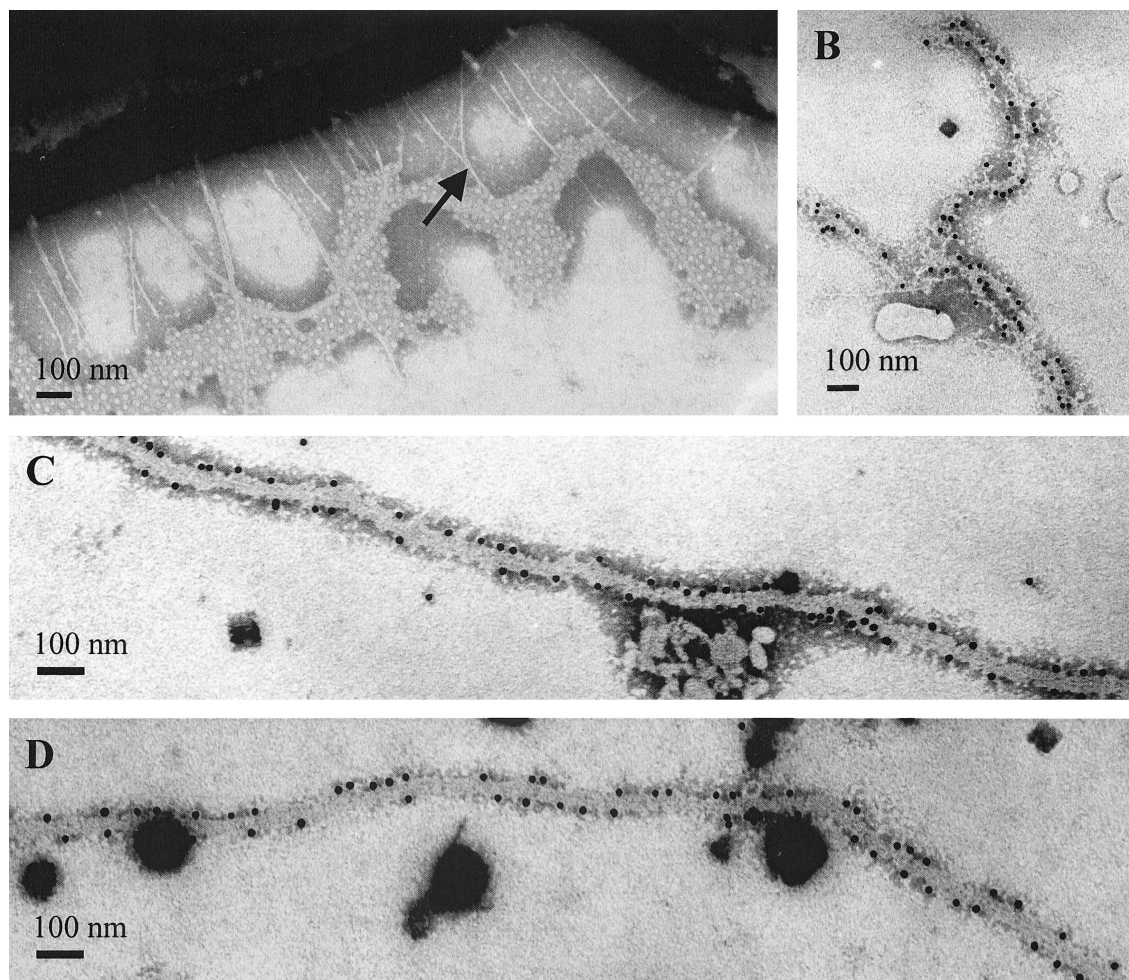
FIG. 1. Electron microscopy of Mat fimbriae. (A) Negatively stained cells of the *fimA sfaA fliC* mutant strain *E. coli* IHE 3034–79 cultivated at 20°C. (B to D) IEM of fimbriae from XL1 Blue MRF′ harboring pMAT3 (B), pMAT9 (C), or pMAT5 (D). Size bars, 100 nm.

IHE 3034 chromosome is available under accession no. AF325731. Identity comparisons were performed with predicted protein sequences of *matB* and the following adhesin genes of *E. coli*: MG1655 *fimA* (9), GenBank accession no. AAC77270; PC31 *fimA* (25), GenBank accession no.X00981; IHE 3034 *sfaA* (15), GenBank accession no. S53211; J96 *papA* (3), GenBank accession no. X03391, K01176, X03392, X61239, and S45610; F17 a-A (32), GenBank accession no. AF022140, M36649, and M62503; MG1655 *ppdD*, GenBank accession no. AE000119 and U00096; K-12 *csgA* (40), GenBank accession no. L04979; C921b-1 *cooA* (44), GenBank accession no. M58550 and M37148; *cooB*, GenBank accession no. X62495; and LMC10 *cooC* and *cooD* (14), GenBank accession no.X76908.

RESULTS

Purification and characterization of fimbriae from the *fimA sfaA fliC* mutant derivative of *E. coli* IHE 3034. The O18acK1H7 meningitis isolate *E. coli* IHE 3034 and the S- and type 1-fimbria-negative double mutant IHE 3034–59 have been described earlier (15, 30, 31, 45, 51). In order to obtain a derivative that is devoid of all surface filaments, the H7 flagellin gene *fliC* of IHE 3034–59 was inactivated by allelic exchange with *fliC::cat::sat*. The *sat* cassette was positioned within the *cat* DNA because it contained a suitable *Nco*I restriction site. Transconjugants with the correct antibiotic markers were assessed for flagellation by agglutination in anti-H7

antiserum, and a nonflagellate mutant was isolated and named IHE 3034–79. The correct genotype of IHE 3034–79 was confirmed by Southern hybridization of *Pvu*I-digested chromosomal DNA by using *fliC* and the *sat* cassette as probes (data not shown). Transmission electron microscopy of IHE 3034–79 cells cultivated at 20°C revealed a lack of flagella, but, unexpectedly, showed the presence of fimbrial filaments on the bacteria (Fig. 1A). The strain IHE 3034–79 was not agglutinated in antisera raised against the type 1 or S fimbriae from the same strain, indicating that the strain expressed a third fimbrial type. This fimbria had a diameter of 5 to 7 nm and was 0.4 to 5 μm in length. Because the expression of this fimbrial type was found to be associated with the major clonal group of MENEC as well as with low temperature (see below), we named it the Mat (meningitis associated and temperature regulated) fimbria.

Mat fimbrial filaments were purified from strain IHE 3034–79 by using deoxycholate and concentrated urea. An SDS-PAGE analysis of the purified Mat fimbriae showed a major peptide with an apparent molecular mass of 18 kDa (Fig. 2A). The apparent molecular size of the Mat fimbrillin was larger than the apparent sizes of the FimA fimbrillin and
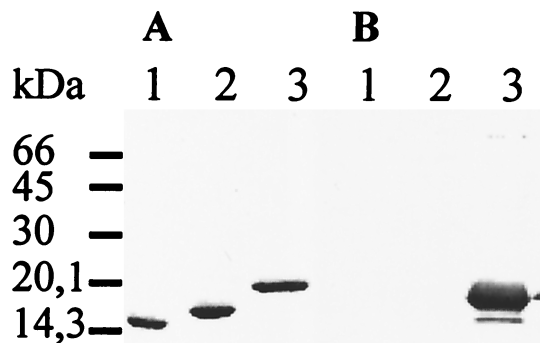
FIG. 2. SDS-PAGE (A) and Western blotting (B) of the S fimbria (lane 1), the type 1 fimbria (lane 2), and the Mat fimbria (lane 3) of *E. coli* IHE 3034. The blotting was done with anti-MAT fimbria antiserum as primary antibodies. The migration distances of molecular mass marker proteins (size in kilodaltons) are indicated on the left.

the SfaA fimbrillin of the same strain (30, 45), which are also shown in Fig. 2A.

Polyclonal antisera against Mat fimbriae purified from *E. coli* IHE 3034–79 and *E. coli* LE392(pMAT1) (see below) were raised in rabbits. In Western blotting, the antibodies detected in the Mat fimbria preparation a major peptide with an apparent molecular mass of 18 kDa as well as a minor peptide with an apparent molecular mass of 16 kDa (Fig. 2B). The antisera against the Mat fimbriae did not recognize the FimA or the SfaA proteins (Fig. 2B). In accordance, ELISA analysis of purified fimbriae showed that the Mat fimbriae did not react with the anti-S fimbria or anti-type 1 fimbria antisera; neither were the type 1 or S fimbriae recognized by the anti-Mat fimbria sera (Table 2). The reactivities of the two anti-Mat fimbriae sera were closely similar.

Amino acid sequencing of the Mat fimbriae gave the N-terminal sequence ADVTAQAVATWSATAKKDTTSKLVV TPLGSLAFQYAEGIK and the internal peptide sequence GLFDVAIEGDSTATAFK. These sequences do not have homologs in the FimA or SfaA sequences, nor did we find any significant homology in any other fimbrial protein sequences deposited in the SWISS-PROT data bank. It was thus concluded that the Mat fimbria represents a novel *E. coli* fimbrial type.

Cloning and sequencing of *mat* genes. We next ligated random 40-kb fragments from the genome of 3034–79 into the cosmid cloning vector pHC79, and transfectants of *E. coli* LE392 expressing the Mat fimbria were identified by bacterial agglutination in anti-Mat fimbria serum and by electron microscopy. A positive recombinant was isolated, and the cosmid was named pMAT1. Cosmid pMAT1 was digested with the *Eco*RI restriction enzyme, and a 7-kb DNA fragment reacting in Southern hybridization with the oligonucleotide probes was identified and cloned into pACYC184, to obtain plasmid pMAT2. Electroporation of pMAT2 into *E. coli* LE392 gave a recombinant strain reacting with the anti-Mat-fimbria antibodies (data not shown).

An extensive homology search revealed that the amino acid sequences obtained from the Mat fimbrillin perfectly matched the predicted amino acid sequence of an open reading frame at nucleotides 17882 to 18469 in the 6- to 8-min region of the *E.*

TABLE 2. Cross-reaction between *E. coli* fimbriae

Specificity of antiserum to *E. coli* fimbria	Titer of antibody to *E. coli* fimbria[a]		
	Type 1	S	Mat
Type 1	4.5	2.4	<2.0
S	2.1	4.6	<2.0
Mat	<2.0	<2.0	4.1

[a] Antibody titer, as tested by the ELISA, is given as the logarithm of the reciprocal of the highest dilution of the antiserum giving an A_{405} of 0.5.

coli K-12 chromosome (5). This DNA encodes a hypothetical protein of 195 amino acids, which was identical to the 57 amino acids sequenced from the Mat fimbrillin. The region surrounding the *mat* homolog at the 6- to 8-min region in the K-12 chromosome encodes six hypothetical proteins, which are located on a 6,841-bp DNA fragment (Fig. 3). Restriction mapping with the enzymes *Nco*I, *Nru*I, *Pst*I, *Pvu*I, *Sac*II, *Sma*I, and *Sna*BI of the 7-kb fragment in pMAT2 gave a pattern closely similar to the one predicted from the K-12 chromosomal sequence (details not shown). We named three of these genes *matA, matB,* and *matC,* because they appeared to affect the synthesis of Mat fimbriae (see below); *matB* encodes the Mat fimbrillin homolog (Fig. 3). The DNA sequence containing the *matA* and *matB* genes of IHE 3034 was determined and compared to the corresponding region in the DNA sequence of MG1655. DNA sequence identity was 93.0% in the 156-bp region upstream of *matA,* 97.0% within *matA,* 93.2% in the 74-bp intergenic region separating *matA* and *matB,* 97.8% within *matB,* and 98.2% in the 57-bp intergenic region between *matB* and *matC.* The *mat* nucleotide sequences are available from the GenBank database under accession no. AF325731.

The predicted amino acid sequence of MatB of IHE 3034 is shown in Fig. 4; it contains a 22-amino-acid signal sequence and differs from the predicted sequence of the MatB homolog of *E. coli* MG1655 only at the position −6, which is within the signal sequence. The calculated molecular mass of mature MatB is 17,853 Da. The predicted MatB sequence of 195 amino acids exhibits 19 to 25% identity with the FimA, SfaA, PapA, F17A, PpdD, CooA, and CsgA fimbrillin sequences, with no areas of significant local identity. The MatB sequence lacks typical motifs identified in *E. coli* fimbrillins. The C terminus of MatB lacks the β-zipper motif recognized by fimbrial chaperones (21), and its signal sequence and N terminus are not related to those in the type IV fimbrillins (53). MatB also lacks cysteine residues and hence disulfide bonds. The predicted MatA sequence is of 196 amino acids, lacks a detectable signal sequence, and contains at residues 145 to 195 a predicted helix-turn-helix DNA-binding motif similar to the consensus pattern in the LuxR family of regulatory proteins (17).

Mutagenesis, complementation, and expression of *matB*. We next inactivated the *matB* gene of *E. coli* IHE 3034 by allelic exchange with a *matB::cat* fusion. A transconjugant with the correct antibiotic markers as well as genotype was isolated and named IHE 3034–90. The correct genotype of IHE 3034–90 was confirmed by Southern hybridization of *Bam*HI-*Sac*II-digested DNA by using *matB* and the *cat* cassette as probes (data not shown). The strain IHE 3034–90 did not express Mat fimbriae, as analyzed by agglutination in anti-Mat
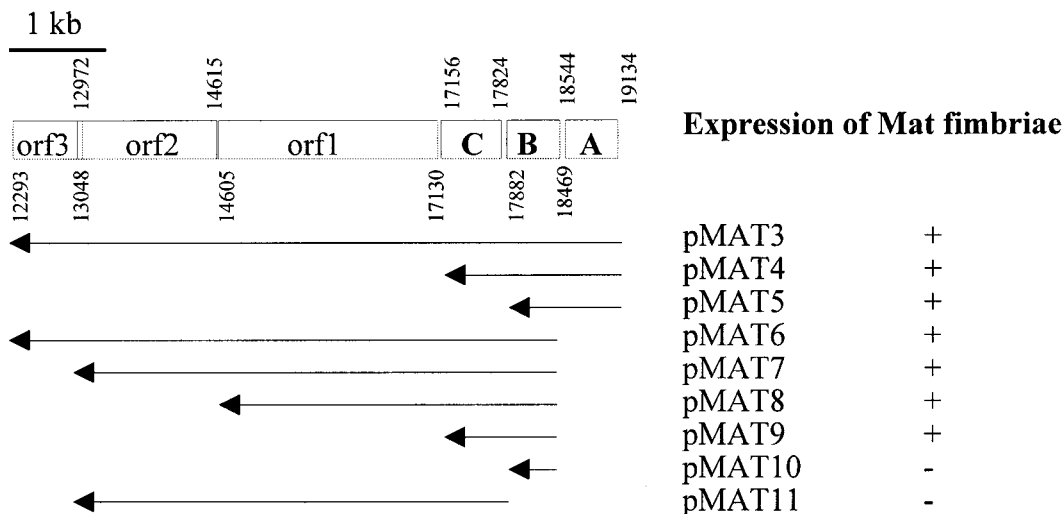
FIG. 3. Gene organization of the 6.8-kb fragment containing *mat* at the 6- to 8-min region in the chromosome of *E. coli* MG1665 (5). The nucleotide numbers refer to the gene location in the sequence available in GenBank (accession no. U73857). Arrows below the sequence indicate DNA fragments cloned into pSE380 and tested for in *trans* complementation of the *matB::cat* mutation in the chromosome of *E. coli* IHE 3034–90 as well as for expression in *E. coli* XL1 Blue MRF′ (on the right).

fimbriae antisera, as well as by IEM (data not shown) and whole-cell ELISA with anti-Mat fimbria antibodies (Fig. 5A).

Most pathogenic *E. coli* strains express more than one fimbrial type, and recently regulatory cross talk between fimbrial operons in *E. coli* was reported (60). We originally detected Mat fimbriae in the *fimA sfaA fliC* triple mutant, which raised the question of whether Mat fimbriae are expressed in the wild-type IHE 3034 as well. Expression levels of Mat fimbriae in IHE 3034 mutants were measured by whole-cell ELISA technology with anti-Mat fimbria antisera as primary antibodies. For screening purposes, the mutations had been introduced into a spontaneous streptomycin-resistant mutant of IHE 3034, IHE 3034-Sm (45), and it appeared that IHE 3034-Sm expressed less Mat fimbriae on the cell surface than did IHE 3034 (Fig. 5A). The mutant strains IHE 3034–2 *fimA*, IHE 3034–8 *sfaA*, IHE 3034–59 *fimA sfaA*, and IHE 3034–79 *fimA sfaA matB* did not exhibit expression levels significantly different from that shown by IHE 3034-Sm, whereas IHE 3034–90 *matB* reacted very poorly with the anti-Mat fimbria antibodies.

A dramatic difference in Mat fimbria expression was seen between cells cultivated in Luria broth at 20 and and those cultivated at 37°C. At the higher temperature, the bacteria exhibited a very low level of Mat fimbria expression, the reactivity with the anti-Mat antibodies being closely similar to that

```
-22                1         11        21
MK KKVLAIALVT VFTGTGVAQA ADVTAQAVAT WSATAKKDTT SKLVVTPLGS
    31         41        51        61        71
   LAFQYAEGIK GFNSQKGLFD VAIEGDSTAT AFKLTSRLIT NTLTQLDTSG
    81         91       101       111       121
   STLNVGVDYN GAAVEKTGDT VMIDTANGVL GGNLSPLANG YNASNRTTAQ
   131        141       151       161       171
   DGFTF S I I S G TTNGTTAVTD YSTLPEGIWS GDVSVQFDAT WTS
```

FIG. 4. Predicted amino acid sequence of MatB of *E. coli* IHE 3034. The sequence has a 22-mer signal sequence. No. 1 indicates the N-terminal amino acid residue of the mature protein. The predicted amino acid sequence from *E. coli* MG1665 (5) contains methionine at the position −6.

seen with the IHE 3034–90 cells (Fig. 5B). Indirect immunofluorescence staining of IHE 3034 grown at 20°C indicated that only a fraction of cells, ranging from 1 to 10% in different cultures, expressed Mat fimbriae on their surface (data not shown). This finding is indicative of phase variation in the Mat fimbria expression (38).
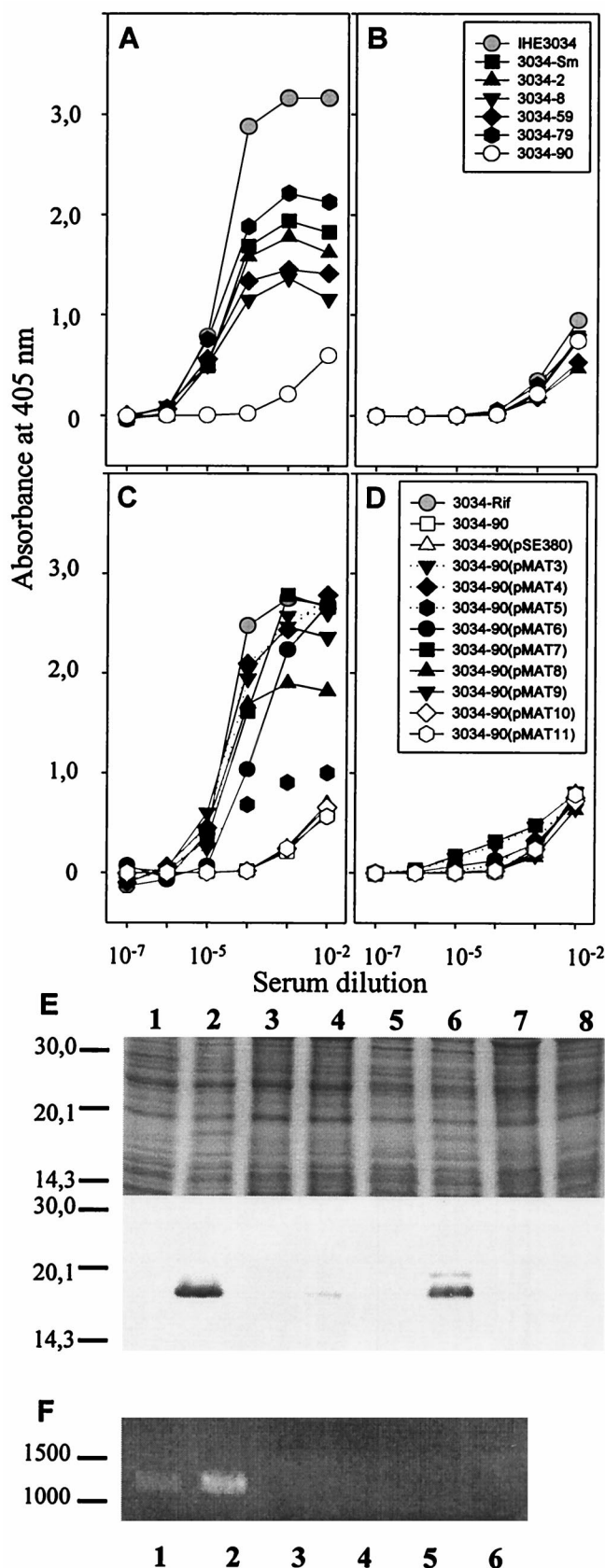
In order to analyze how many genes of the putative *mat* gene cluster are needed for Mat fimbria expression, the *matB::cat* mutation on the IHE 3034–90 chromosome was complemented in *trans* with pSE380 containing different fragments of the *matB*-containing region of pMAT2 (Fig. 3). The PCR cloning was done on the basis of the MG1655 sequence, so that in the pSE380 derivative, the *mat* genes were under the inducible *trc* promoter. The expression of the Mat fimbria was assessed with bacteria grown at 20°C by agglutination, whole-cell ELISA, and IEM with anti-Mat fimbria antibodies. Complementation with pMAT11 lacking *matB* did not restore Mat fimbria formation (Fig. 3), nor did the cells react in whole-cell ELISA (Fig. 5C) or in agglutination with the anti-Mat-fimbria antibodies (data not shown). Similarly, complementation with *matB* alone in pMAT10 did not restore the surface expression of the Mat fimbriae (Fig. 3 and 5C). A low level of Mat expression was detected in *E. coli* IHE 3034–90(pMAT5) complemented with the *matA-B* DNA, and a higher expression level was seen in cells complemented with pMAT9 containing *matBC* and with pMAT8 containing *matB-orf1*. The expression levels by the other complementation derivatives did not differ significantly from each other (Fig. 3 and 5C). Expression of the same pMAT plasmids in *E. coli* XL1 Blue MRF′ gave essentially similar results (data not shown). IEM did not reveal any major differences in the morphology or immunological reactivity of the fimbriae of *E. coli* harboring the pMAT plasmids: examples with pMAT3, pMAT 5, and pMAT9 are shown in Fig. 1.

A striking feature of the complementation assays detected with *E. coli* IHE 3034–90 was that the amount of Mat fimbriae

expressed on the surface of the complemented strains grown at 37°C was greatly decreased, as analyzed by whole-cell ELISA (Fig. 5D). We also analyzed by Western blotting the amount of MatB in cells of *E. coli* IHE 3034–90(pMAT3) and XL1 Blue MRF′(pMAT3) grown at 37 and 20°C. MatB was detected in cells grown at 20°C, but only in trace amounts or not at all in cells grown at 37°C (Fig. 5E). As a control, we expressed under the *trc* promoter in pSE380 the *pla* gene (pMRK1) encoding an outer membrane protease. Analysis of expression of Pla in IHE 3034–90 by Western blotting showed expression at similar amounts at both temperatures (data not shown).

We used RT-PCR to analyze whether transcription of *matB* and *matC* is affected by temperature. DNA fragments of the expected sizes of *matB* (not shown) and *matBC* (Fig. 5F) were amplified from RNA of IHE 3034 cells grown at 20°C, and the *matBC* signal was weaker by 70% in cells grown at 37°C. No *matB-C* transcripts were detected in MG1665 or IHE 3034–90 cells at either temperature (Fig. 5F). A *matA* transcript was detected in all three strains grown at both temperatures, whereas PCR amplification with primer specific for *matAB* or *matAC* did not produce detectable amplimers (data not shown).

In order to detect other environmental signals besides low temperature that might influence expression of the Mat fimbria, we assessed Mat expression by *E. coli* strains IHE 3034, LE392, and MG1655 grown at 37°C under different conditions. The conditions included cultivation in the presence of 5% (vol/vol) CO_2 atmosphere, 0.2% (wt/vol) glucose, 0.2 mM iron chelator 2,2-dipyridyl, low (0.1%) or high (2%) concentrations of NaCl, or 10% (vol/vol) human blood, as well as anaerobic conditions. None of these conditions induced detectable Mat fimbria expression.

Presence of *matB* and expression of the Mat fimbria in isolates of *E. coli*. IHE 3034 belongs to the serogroup O18acK1H7, which forms the major clonal group of MENEC. These isolates form a homogenous and distinct group of *E. coli*, as analyzed by several phenotypic assays and isoenzyme polymorphism (30, 31, 51). We analyzed 27 isolates representing various pathogroups of *E. coli* (Table 1) for the presence of *matB* as well as expression at 20 and 37°C of the Mat fimbria. The analyzed strains belonged to the O18K1H7 MENEC

FIG. 5. Expression of Mat fimbriae in *E. coli*, as analyzed by whole-cell ELISA with anti-Mat fimbriae as primary antibodies. (A and C) Expression in cells grown at 20°C. (B and D) Expression in cells grown at 37°C. (A and B) Expression is shown for the wild-type IHE 3034, the spontaneous streptomycin-resistant strain IHE 3034-Sm, the *fimA*::*cat* derivative IHE 3034-2, the *sfaA*::Gm derivative IHE 3034-8, the *fimA*::*cat sfaA*::Gm double mutant IHE 3034-59, the *fimA*::*cat sfaA*::Gm *fliC*::St triple mutant IHE 3034-79, and the *matB*::*cat* derivative IHE 3034–90. (C and D) Complementation of the *matB*::*cat* mutation in IHE 3034–90 by the pMAT-plasmids encoding regions of the putative *mat* gene cluster. (E) SDS-PAGE (top) and Western blotting (bottom) of cellular proteins in *E. coli* XL1 Blue MRF-′(pSE380) (lanes 1 and 3), *E. coli* XL1 Blue MRF′(pMAT3) (lanes 2 and 4), *E. coli* IHE 3034–90(pSE380) (lanes 5 and 7), and *E. coli* IHE 3034–90(pMAT3) (lanes 6 and 8). The bacteria were cultivated at 20°C (lanes 1, 2, 5, and 6) or at 37°C (lanes 3, 4, 7, and 8). (F) RT-PCR of RNA from *E. coli* IHE 3034 (lanes 1 and 2), MG1665 (lanes 3 and 4), and IHE 3034–90 (lanes 5 and 6). The bacteria were cultivated at 37°C (lanes 1, 3, and 5) or 20°C (lanes 2, 4, and 6). Amplification was done with a primer pair specific for *matBC*.

clonal group or to the clonal groups of UPEC (O1K1H7, O4K12H7, O6K2H1, O18K5H7, and O6K13H1), or they represented the diarrhea-causing pathogroups EAEC, EHEC, EPEC, and ETEC. Additionally, K-12 strains and the widely used UPEC strain 536 were analyzed. PCR and Southern hybridization showed the presence of a *matB* homolog in all strains except the two ETEC isolates (Table 1). The apparent size of the amplified *matB* fragment in the isolates was invariant and equalled the one seen in IHE 3034 (data not shown). However, expression of the Mat fimbria was detected only in the MENEC isolates grown at 20°C (Table 1).

Hemagglutination by *E. coli* IHE 3034–79. Many fimbrial types of *E. coli* recognize specific carbohydrate sequences. In order to test whether this might be the case for the Mat fimbria as well, we assessed hemagglutination of human erythrocytes by the strain IHE 3034–79 cultivated at 20°C. No agglutination was detected; neither did enzymatic treatments known to reveal cryptic carbohydrates on the erythrocyte surface induce detectable hemagglutination.

DISCUSSION

Mat fimbria represents a novel type of *E. coli* fimbria that is morphologically similar to the type 1 or P fimbriae, but serologically and in a subunit sequence unrelated to the previously characterized fimbrial proteins of *E. coli* or other bacteria. Although the *matB* gene encoding the major subunit was found to be common in *E. coli* isolates, it was expressed on the bacterial surface only in the genetically conserved clonal group of O18acK1H7 MENEC cultivated at low temperature. The *matB* gene is the fourth fimbrillin gene detected in laboratory K-12 strains, which previously have been shown to harbor *fim* genes encoding the type 1 fimbria, the *csg* genes encoding curli, as well as *ppdD* encoding the type IV fimbrillin (5, 9,25, 40, 48). Of these, only the type 1 fimbriae are expressed by *E. coli* K-12 under routine laboratory conditions at 37°C. Curli expression takes place at low temperature and low osmolarity (40). It was recently found that a fraction of *E. coli* isolates from cases of human adult urosepsis also express curli at 37°C (4). The type IV fimbriae require an exogenous secretion apparatus for surface expression in *E. coli* K-12 (49), but are expressed in EPEC that harbor the EPEC adherence factor plasmid (35, 36). The *matB* fimbrillin gene appears to be common in *E. coli* and is expressed at low temperature by isolates of a genetically distinct pathogroup.

matB encodes the major subunit of the Mat fimbria. The complete nucleotide sequence of *matB* was determined and the deduced protein sequence matched the sequence of 57 amino acids obtained from the Mat fimbria. The knockout *matB*::*cat* mutation abolished Mat fimbria production, which was complemented in *trans* by *matB*-containing DNA together with at least either *matA* or *matC*. The predicted sequence of MatB was not related to the fimbrillin sequences deposited in data banks and lacks the N-terminal motifs of type IV fimbrillins (53) and CsgA-related fimbrillins (12, 40). MatB also lacks cysteine residues and the C-terminal β-zipper motif of chaperone-assisted fimbrillins (21). These characteristics are also lacking in the CooA fimbrillin of the CS1 filaments and in the other fimbrillins detected on human ETEC strains (46). The fimbrillins on human ETEC share 40 to 50% sequence identity with each other, and their plasmid-borne gene clusters are

closely related in gene organization (46). However, we found only 23% sequence identity between MatB and CooA, and the *matA* and *matC* gene products showed a similar low sequence identity with the four Coo proteins of the CS1 fimbria (46). The ELISAs, Western blotting, and IEM with anti-Mat fimbria antibodies showed that the Mat fimbria was immunologically distinct from the type 1 and S fimbriae expressed by MENEC. We thus conclude that the Mat fimbria represents a novel fimbrial type of *E. coli*. We could not detect any carbohydrate binding by the Mat fimbria and are currently analyzing the binding of Mat fimbria to epithelial cells and tissue proteins.

A *matB* homolog was detected in 25 of the 27 *E. coli* strains analyzed in this study. The analyzed isolates represented major pathogroups of *E. coli* as well as K-12 strains; the two *matB*-negative strains were ETEC isolates. The nucleotide sequence of *matB* of IHE 3034 was 97.8% identical to the *matB* from MG1665, and the *matB* homologs amplified from the genomic DNA of the *E. coli* isolates were of invariable size, also in isolates found not to express MatB. These findings indicate conservation of the *matB* gene in *E. coli*. We detected Mat fimbria expression only in the O18acK1H7 clonal group of MENEC, and it is noteworthy that the O1K1H7 isolates, which on the basis of isoenzyme polymorphism form a clonal group of *E. coli* strains genetically closest to the O18acK1H7 MENEC group (43, 51), did not express their *matB* gene under the conditions we tested.

We subcloned *matB* from IHE 3034–79 on a 7-kb fragment that supported Mat fimbria expression when transformed into *E. coli* K-12. This DNA fragment has a homolog in the MG1665 chromosome at the 6- to 8-min region and contains six putative genes (5), of which we found *matA*, *matB*, and *matC* affected Mat fimbria formation. The two chromosomal regions are highly similar; nucleotide sequencing showed that the *matAB* region was 96.6% identical for the sequenced 1,471-bp fragment, and a high level of identity of the whole 7-kb region was also evident in the restriction mapping. At present, the number of genes that are involved in Mat fimbria sythesis and regulation remains open. We also do not know whether genes located at a distance from the *matB* region are involved in fimbria synthesis. The fimbrial gene clusters of *E. coli* usually contain four or more genes needed for the structure, assembly, or regulation of the fimbrial filament (reviewed in references 20 and 27). Our complementation assays indicated that *matB* alone is not sufficient to confer Mat fimbria expression on *E. coli* XL1 or the strain IHE 3034–90 with the polar *matB*::*cat* mutation. Complementation was slightly more efficient in the *matAB* transformant and further increased in the *matBC* transformant, but was not significantly enhanced when fragments encompassing more than the *matBC* genes were introduced into IHE 3034 *matB*::*cat* or XL1 Blue MRF′. The RT-PCR analysis indicated that *matB* and *matC* are part of the same transcript, whereas we did not detect a *matABC* transcript, which suggests that *matA* is transcribed independently from *matBC*. It is noteworthy that the *E. coli* strain MG1665 contained a *matA* transcript, but we failed to amplify a *matBC* RNA from this strain. IEM did not reveal any major structural differences between Mat fimbrial filaments on *E. coli* K-12 expressing the pMAT plasmids. Western blotting of the Mat fimbriae showed the presence of a minor protein apparently smaller than MatB; its size, however, did not match those

of any of the gene products from the *mat* region, and it may be a degradation product from MatB. On the other hand, in *trans* expression of *mat* genes in IHE 3034–90(pMAT3) showed a minor protein with an apparent size of ca. 20 kDa, which is close to the calculated size of mature MatC (22.4 kDa). The function of MatC in fimbria formation remains open. Lack of the chaperone target motif in the C terminus of MatB suggests that Mat fimbriae are not assembled by the classical chaperone-usher pathway of the P and the type 1 fimbriae (20), and the assembly mechanism of the Mat fimbria remains to be elucidated. The predicted sequence of MatA does not contain a signal sequence, whereas a putative signal sequence is present in the *matB* and *matC* gene products. The predicted MatA sequence contained a C-terminal helix-turn-helix DNA-binding motif of the LuxR family of regulatory proteins (17). This indicates that MatA may be a cytoplasmic protein functioning in regulation.

We found that transcription of *matBC* in IHE 3034 cells was reduced but not abolished at 37°C and that expression of *mat* genes from the plasmid pMAT3 with the inducible *trc* promoter also was temperature sensitive. These findings indicate that the temperature regulation of the *mat* genes involves transcriptional as well as posttranscriptional events. Expression of curli in *E. coli* K-12 is favored at low temperatures. The transcription of *csgA* is positively regulated by the RpoS sigma factor and repressed by the histone-like protein H-NS, but the temperature and osmolarity regulation of curli expression involves other factors (40). The mechanisms of the temperature regulation of Mat expression remain to be determined. Temperature is known to regulate translation by affecting mRNA conformation (2, 18) or the translational machinery itself (reviewed in reference 22). The *matA* sequence contains four copies of putative RNase E target sequences (37), which might become accessible upon temperature-induced conformational change. Temperature may also affect the stability of the MatB protein. The mechanisms behind the repression of *mat* expression in most *E. coli* isolates also remain open. We found by DNA sequencing and restriction mapping that the 7-kb *mat* regions in IHE 3034 and MG1655 are highly similar, which indicates that the lack of Mat fimbria expression in *E. coli* MG1655 does not result from truncation or lack of genes at this chromosomal region. *E. coli* IHE 3034 and another O18acK1H7 MENEC isolate were recently found to be *rpoS* mutants (57); whether this holds true for the entire O18acK1H7 MENEC clone and how RpoS influences *mat* expression are to be elucidated. RpoS is a positive regulator of curli expression, and we indeed have not detected curli expression in IHE 3034 cells cultivated at 20°C (B. Westerlund-Wikström and R. Pouttu, unpublished data). Our ongoing work has shown that silencing of *rpoS* does not alone lead to expression of the Mat fimbria. The regulation of the Mat fimbria expression appears complex and is particularly interesting because it seems to be a clonal property in *E. coli*.

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