Mat fimbriae promote biofilm formation by meningitis-associated *Escherichia coli*

Timo A. Lehti,1 Philippe Bauchart,2 Johanna Heikkinen,1 Jörg Hacker,2,3 Timo K. Korhonen,1 Ulrich Dobrindt2 and Benita Westerlund-Wikström1

1General Microbiology, Department of Biosciences, FI-00014 University of Helsinki, Finland
2Institute for Molecular Infection Biology, University of Würzburg, D-97080 Würzburg, Germany
3Robert Koch-Institute, D-13353 Berlin, Germany

The mat (or ecp) fimbrial operon is ubiquitous and conserved in *Escherichia coli*, but its functions remain poorly described. In routine growth media newborn meningitis isolates of *E. coli* express the meningitis-associated and temperature-regulated (Mat) fimbria, also termed *E. coli* common pilus (ECP), at 20 °C, and here we show that the six-gene (matABCDEF)-encoded Mat fimbria is needed for temperature-dependent biofilm formation on abiotic surfaces. The matBCDEF deletion mutant of meningitis *E. coli* IHE 3034 was defective in an early stage of biofilm development and consequently unable to establish a detectable biofilm, contrasting with IHE 3034 derivatives deleted for flagella, type 1 fimbriae or S-fimbriae, which retained the wild-type biofilm phenotype. Furthermore, induced production of Mat fimbriae from expression plasmids enabled biofilm-deficient *E. coli* K-12 cells to form biofilm at 20 °C. No biofilm was detected with IHE 3034 or MG1655 strains grown at 37 °C. The surface expression of Mat fimbriae and the frequency of Mat-positive cells in the IHE 3034 population from 20 °C were high and remained unaltered during the transition from planktonic to biofilm growth and within the matured biofilm community. Considering the prevalence of the highly conserved mat locus in *E. coli* genomes, we hypothesize that Mat fimbria-mediated biofilm formation is an ancestral characteristic of *E. coli*.

INTRODUCTION

*Escherichia coli* is a multifaceted bacterium that colonizes the mammalian intestine as a harmless commensal but also causes a repertoire of intestinal and extraintestinal infectious diseases (Dobrindt, 2005) and survives in the environment (Savageau, 1983). *E. coli* from the mother and/or the surrounding environment are generally among the earliest colonizers in the oxygenous neonatal gut. During the first months of life a succession of bacterial populations progresses to a complex, more stable and adult-like microbial community dominated by strict anaerobes (Adlerberth & Wold, 2009). However, some *E. coli* strains are able to persist as a member of the normal microbiota and constitute a major portion of the facultative intestinal flora on adult mucosal surfaces. *E. coli* also frequently colonizes anatomical locations outside the gastrointestinal tract, e.g. vagina and urinary bladder (Obata-Yasuoka et al., 2002; Rosen et al., 2007). The population structure of *E. coli* is largely clonal (Ochman & Selander, 1984; Selander et al., 1986), and isolates are frequently categorized into distinct pathogroups according to specific combinations of phenotypic traits. Based on multilocus enzyme electrophoresis, *E. coli* strains fall into four main phylogenetic groups (designated A, B1, B2 and D), each containing varying proportions of different pathogroups and non-pathogens. *E. coli* strains resident in childhood and adult microbiota commonly include pathogenic variants (Sarff et al., 1975; Sittonen, 1992), predominantly belonging to the virulence-associated phylogenetic group B2 (Nowrouzian et al., 2005; Obata-Yasuoka et al., 2002; Zhang et al., 2002). Despite their primarily asymptomatic colonization, these B2 strains are able to induce various diseases, such as neonatal meningitis, urinary tract infections, sepsis and pneumonia, after entering into extraintestinal sites in both normal and compromised hosts (Picard et al., 1999; Russo & Johnson, 2000). This implies that the normal flora is an important reservoir for extraintestinal pathogenic *E. coli* strains.

The evolutionary success of *E. coli* relies in part on the ability to express surface proteins that mediate cellular interactions on biotic and abiotic surfaces. Expression of meningitis-associated and temperature-regulated (Mat) fimbriae was originally detected in the genetically conserved O18ac : K1 : H7 clonal group of human newborn meningitis...
**E. coli** (NMEC) isolates cultivated at low temperature (Pouttu et al., 2001). Recently, expression of the fimbrria, also called **E. coli** common pilus (ECP; Rendón et al., 2007), was found to be widespread in pathogenic and non-pathogenic **E. coli** strains and to contribute, to a variable extent, to **E. coli** adherence onto cultured epithelial cells (Blackburn et al., 2009; Lasaro et al., 2009; Rendón et al., 2007; Saldaña et al., 2009); however, the adhesin or the receptor have not yet been identified. The *mat* (or *ecp*) genes are widespread and conserved in **E. coli**, and it appears that their regulatory mechanisms are modulated by multiple environmental cues and differ among clonal groups of **E. coli** (Blackburn et al., 2009; Pouttu et al., 2001; Rendón et al., 2007).

A matrix-enclosed, organized-community lifestyle in a biofilm is a common mode of growth for many bacterial species that inhabit diverse environments (Hall-Stoodley et al., 2007). Biofilm formation allows bacteria to survive in stressful conditions and facilitates transmission to new niches. **E. coli** have developed a spectrum of sophisticated and finely controlled pathways to promote growth in such surface-bound communal structures (Beloin et al., 2008; Van Houdt & Michiels, 2005). The early stages of biofilm development often involve employment of flagella-mediated motility for reversible and initial attachment onto a surface (Pratt & Kolter, 1998; Wood et al., 2006). Production of other surface organelles, including F pilus, type 1 fimbrriae and curli, is needed for irreversible attachment to strengthen the adhesion (Ghigo, 2001; Pratt & Kolter, 1998; Reisner et al., 2003; Vidal et al., 1998). In this study, we analysed the *mat* cluster of NMEC for its contribution to biofilm formation and describe a function of Mat fimbrriae as a biofilm-promoting factor.

## METHODS

**Strains, plasmids and growth conditions.** **E. coli** strains and plasmids used in this study are listed in Table 1. All cultivations were performed without agitation at 20 or 37 °C in Luria–Bertani (LB) broth or in M63 medium [minimal M63 salts (Pardee et al., 1959) supplemented with 0.4% glucose and 1% Casamino acids]. When necessary, media were supplemented with ampicillin 100 µg ml<sup>-1</sup>.

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

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype or description</th>
<th>Source or reference</th>
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<td><strong>E. coli</strong> strains</td>
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<tr>
<td>IHE 3034-Rif</td>
<td>Rifampicin-resistant IHE 3034, O18:K1:H7</td>
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chloramphenicol 25 μg ml⁻¹, gentamicin 30 μg ml⁻¹, rifampicin 75 μg ml⁻¹, streptomycin 100 μg ml⁻¹, streptomycin 100 μg ml⁻¹ or tetracycline 12.5 μg ml⁻¹. Induction of mat genes in recombinant E. coli strains was done with 5 μM IPTG.

**Construction of chromosomal mutants.** In-frame deletion of the entire matBCDEF region and each putative mat gene on the chromosome of the spontaneously Rif-resistant strain IHE 3034-Rif was performed by site-specific mutagenesis with the pir-dependent suicide vector pCVD442 essentially as described by Mobley et al. (1993). Briefly, the 5' and 3' flanking sequences of each deletion site were amplified by PCR from IHE 3034 chromosomal DNA with Pfu polymerase (Promega), fused with each other by recombinant PCR and then inserted between the SacI and XbaI sites of the pCVD442. The resulting plasmids were introduced into IHE 3034-Rif by conjugation using the donor strain S17-1 matBCDEF derivative, as described above. The matB gene was deleted from the Sm-resistant strain IHE 3034-79, a fimA sfaA fliC matB quadruple mutant, the matB gene was deleted from the Sm-resistant strain IHE 3034-Sm, and the nucleotide sequences were verified by sequencing. Plasmid construct pMAT20 was similarly made by cloning matBCDEF genes from MG1655 chromosomal DNA into pSE380, and confirmed by partial sequencing. The plasmids were introduced into IHE 3034-Rif by electroporation (Sambrook & Russell, 2001).

In the matBCDEF mutant strain, the deletion was complemented by insertion of the wild-type alleles back into the chromosomal deletion site by reverse allelic exchange using pCVD442.

**Cloning of mat genes.** For in trans complementation of the individual mat deletion derivatives, plasmids encoding MatC, MatD, MatE and MatF were constructed in the inducible pSE380 vector as described earlier (Pouttu et al., 2001). Briefly, the genes were amplified by PCR from IHE 3034 chromosomal DNA with Pfu polymerase (Promega), cloned into the inducible trc promoter, and the nucleotide sequences were verified by sequencing. Plasmid construct pMAT20 was similarly made by cloning matBCDEF genes from MG1655 chromosomal DNA into pSE380, and confirmed by partial sequencing. The plasmids were introduced into the different E. coli strains by electroporation (Sambrook & Russell, 2001).

**Biofilm formation.** Biofilm formation on plastic surfaces was measured using the microtitre plate assay system, performed basically as described by Genevaux et al. (1996). Briefly, the cells were grown for 48 h in M63 medium at 20 or 37 °C, diluted in M63 medium (1:200, v/v) and then 160 μl of each suspension was added into the wells of a PVC microtitre plate (Falcon). After 48 h of growth at 20 or 37 °C, adherent cell layers were washed twice with PBS, pH 7.1, dried, stained with 0.1 % crystal violet (CV) for 10 min and again washed twice with PBS and dried. To quantify biofilm formation the CV stain was solubilized in ethanol/acetone (80:20, v/v), diluted 1:4 in ethanol/acetone (80:20, v/v) and the absorbance at 595 nm was measured in an ELISA recorder (Multiskan EX, Thermo Scientific). The mean absorbance from six wells was calculated for each assay. We also used this method to quantify biofilm formation on polystyrene microtitre plates (Nunc) and glass coverslips (Menzel-Gläser).

**Initial attachment.** To assess the initial attachment of IHE 3034-Rif derivatives onto PVC surfaces, the strains were grown in static M63 medium for 48 h at 20 °C, diluted in M63 to an OD₆₀₀ value of 0.1, and then 500 μl of each suspension was added to a 24-well plate containing sterile PVC tabs (1 x 1 cm²) and incubated at 20 °C. After various time intervals the tabs were rinsed twice with PBS, fixed with 3.5 % paraformaldehyde in PBS for 10 min, immunostained (see below) and examined by epifluorescence microscopy (Olympus BX50). The numbers of bacteria in 20 randomly chosen microscopic fields of 10⁵ μm² were determined. The initial attachment to PVC was also tested by using the CV-based method of O'Toole & Kolter (1998), with the following modifications. The strains were grown statically in M63 medium for 48 h at 20 °C, diluted in fresh M63 to an OD₆₀₀ value of 0.1 or 1.0, and 160 μl of each suspension was inoculated into the wells of a 96-well microtitre plate. After incubation for 1 h at 20 °C, the adhered cells were washed twice with PBS and quantified by CV staining, as described above.

**Detection of Mat fimbriae expression by whole-cell ELISA.** Polyclonal rabbit antiserum against Mat fimbriae of E. coli was available from previous work (Pouttu et al., 2001). Bacteria were grown statically in glass flasks as planktonic cultures and collected by centrifugation. In order to investigate the effect of biofilm growth on Mat fimbriae expression, cells were also grown in the wells of PVC microtitre plates. The non-attached planktonic cells were first removed from the wells (preferred to as surface-influenced planktonic (SIP) cells), and surface-attached biofilm cells were harvested from the wells after washing by scraping cells from the surface and resuspending them in PBS. The amount of Mat fimbriae on bacterial cells was assessed by whole-cell ELISA according to the procedure of de Ree et al. (1986). Briefly, bacterial cells were adjusted to equal concentrations by resuspending in PBS to an OD₆₀₀ value of 0.25, and microtitre plates (Nunc) were coated with 100 μl of the bacterial suspension per well and dried overnight at 37 °C. The plates were then washed three times with PBS containing 0.05 % Tween 20 (PBS-T) and wells were blocked with 100 μl of PBS containing 2 % BSA at room temperature for 2 h. After washing three times with PBS-T, 100 μl of anti-Mat fimbria antibodies diluted from 10⁻² to 10⁻⁷ in PBS containing 1 % Tween 20 (PBS-T2) was added to wells in duplicate and incubated at 37 °C for 2 h. The plates were then washed three times with PBS-T, and 100 μl of alkaline phosphatase-conjugated swine anti-rabbit immunoglobulins (Dako) diluted 1:750 in PBS-T2 was added and incubated at room temperature for 2 h. Finally, the plates were washed three times with PBS-T, 100 μl phosphatase substrate (Sigma) at 1 mg ml⁻¹ in diethanolamine/magnesium chloride buffer (Reagen) was added and the absorbance at 405 nm of each well was measured after 30 min incubation at 37 °C by an ELISA recorder (Multiskan EX, Thermo Scientific).

**Indirect immunofluorescence.** For detection of Mat fimbriae expression by indirect immunofluorescence as described by Pouttu et al. (2001), bacterial cells were suspended in PBS and immobilized on glass slides, fixed with 3.5 % paraformaldehyde in PBS for 10 min, washed and left to react for 30 min with anti-Mat fimbria antibodies diluted 1:50 in PBS containing 1 % BSA. After washing three times with PBS, the slides were incubated for 30 min with Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen) diluted 1:1000 in PBS containing 1 % BSA. After washing and mounting in Fluorescent Mounting Medium (Dako), the slides were examined in an epifluorescence microscope (Olympus BX50) equipped with a filter for FITC. The proportions of Mat-positive bacteria in six randomly chosen microscopic fields of 10⁵ μm² were determined.

**Western blotting.** Bacterial cells were adjusted to equal concentrations by resuspending in PBS to an OD₆₀₀ of 0.8. Pelleted cells from 750 μl of the bacterial suspension were resuspended in 20 μl H₂O, HCl-treated with 2 μl 1 M HCl, boiled for 2 min and neutralized with 2 μl 1 M NaOH. Denatured samples were mixed with half of their volume of SDS-PAGE loading buffer and boiled for 5 min. Samples of 15 μl were subjected to 12 % SDS-PAGE, transferred onto a PVDF membrane (GE Healthcare) and then Mat fimbrial proteins were
reacted with primary anti-Mat fimbria antibodies (diluted 1:500 in PBS containing 0.1% Tween 20), followed by the secondary peroxidase-conjugated anti-rabbit IgG (1:2500; GE Healthcare), and enhanced chemiluminescence detection reagents (GE Healthcare) according to the manufacturer’s instructions. To visualize the bands, the membrane was exposed to X-ray film (Agfa).

**Serum agglutination.** Bacterial agglutination in anti-Mat antiserum, diluted 10⁻² in PBS, was performed on glass slides by a routine procedure (Rhen et al., 1983).

### RESULTS AND DISCUSSION

**Effects of non-polar mutations on Mat expression**

The chromosomal 7 kb mat gene cluster of O18ac:K1:H7 meningitis isolate *E. coli* IHE 3034 contains six putative genes, of which *matA*, *matB* and *matC* have earlier been shown to affect Mat fimbria biogenesis (Pouuttu et al., 2001). *matB* (orthologues also known as *yagZ* and *ecpA*) encodes the major fimbriinn, which is the only known structural element of the Mat fimbria (Pouuttu et al., 2001). To define more precisely the genes of the mat cluster that participate in Mat fimbria biogenesis, we inactivated separately, by in-frame deletions, the entire matBCDEF region and each of these putative mat genes of the spontaneously Rif-resistant strain IHE 3034-Rif. Whole-cell ELISA using polyclonal anti-Mat fimbria antiserum as primary antibodies showed a decreased surface expression of Mat fimbriae in the deletion mutants cultivated at 20°C (Fig. 1a). As a negative control for Mat fimbria expression, the analysis included the *matB*::cat derivative of IHE 3034-Rif (Pouuttu et al., 2001). Plasmids encoding MatB, MatC, MatD, MatE and MatF (Table 1) were used to complement the corresponding mat deletions, and the matBCDEF mutant strain was complemented by insertion of the genes back into the chromosomal deletion site. After growth at 20°C, each complemented strain showed a Mat expression level comparable to that of the wild-type strain (Fig. 1b) confirming that the individual mutations had no polar effects on expression. No Mat fimbriae were detected in bacteria grown at 37°C (Fig. 1a, b, lower panels). These results indicated that the expression of the matB–F genes is required for Mat fimbria biogenesis in *E. coli* IHE 3034, in addition to *matA*, which was shown by Pouuttu et al. (2001) to be required for synthesis of Mat fimbriae.

**Biofilm formation by meningitis *E. coli* IHE 3034 involves Mat fimbriae**

The capacity of strain IHE 3034-Rif and its derivatives to form a monospecies biofilm on PVC plastic surfaces was quantified using CV staining of bacteria after 48 h static incubation in the biofilm-promoting M63 medium at 20 or 37°C. High biofilm-forming ability is associated with asymptomatic bacteriuria *E. coli* strains (Ferrière et al., 2007); therefore the test included for comparison the strain ABU38, which in our previous work has proven to be efficient in biofilm formation (unpublished). The commensal-like *E. coli* strain ABU38 belongs to phylogenetic lineage B1, has few virulence-associated genes and is unable to express type 1 fimbriae (Zdzierski et al., 2008). This strain also differs from strain IHE 3034 in Mat fimbriae expression, since no agglutination was detected with anti-Mat fimbria antiserum. The biofilm analysis demonstrated a strong biofilm formation by IHE 3034-Rif after growth at low temperature, but not at 37°C, in contrast to the phenotype of ABU38 (Fig. 2a). Thus the mechanisms of biofilm production by the group B2 strain IHE 3034-Rif and the group B1 strain ABU38 are different. No biofilm was formed by the Mat fimbriae-deficient matBCDEF mutant strain (Fig. 2a, b) nor the individual mat deletion mutant strains (Fig. 2a). The apparent differences in biofilm-forming abilities are not due to the differential growth in the M63 medium, as the mutants exhibited growth rates comparable to that of the parent strain (data not shown). As shown in Fig. 2(a), single-copy chromosomal complementation of the matBCDEF deletion completely restored the biofilm-forming phenotype. When biofilm formation by IHE 3034-Rif was quantified over time at 20°C, a steady plateau was seen after 24 h of incubation, while ABU38 had already formed the highest-density biofilm at 16 h and diminished thereafter (Fig. 2b). Parallel results were obtained when polystyrene was used as a template instead of PVC, whereas IHE 3034-Rif
developed biofilm poorly on glass surfaces (data not shown), implying that biofilm formation by IHE 3034-Rif is dependent on substratum hydrophobicity. Our results are in line with the recent observation that Mat fimbriae do not mediate biofilm formation on glass surfaces at 37°C in the probiotic E. coli Nissle 1917 strain (Lasaro et al., 2009).

The mat operon in the genome of E. coli MG1655 (K-12) is 97.8% identical in sequence to that in the IHE 3034 genome, but MG1655 is incapable of Mat fimbriae expression in LB (Pouttu et al., 2001) or M63 medium (Fig. 2d). As expected, strain MG1655 had no ability to form a biofilm under the growth conditions employed in this study (Fig. 2c). To assess whether induced production of Mat fimbriae promotes formation of biofilm in the non-NMEC host MG1655, we introduced the plasmid pMAT6, encoding Mat fimbriae from IHE 3034, into MG1655. The overexpression from the plasmid resulted in the production of Mat fimbriae at both 20 and 37°C (Fig. 2d) and biofilm growth at low temperature (Fig. 2c).

Comparison of the mat cluster sequence from MG1655 with IHE 3034 shows several non-synonymous nucleotides and hence amino acid variation in the predicted Mat proteins. Previous studies have demonstrated that the minor allelic variation of the type 1 fimbrial adhesin FimH affects tissue tropism through changes in the mannose receptor specificity (Pouttu et al., 1999; Sokurenko et al., 1995, 1998) and influences the ability to promote mannose-sensitive biofilm formation (Schembri & Klemm, 2001). These findings prompted us to test the functional properties of the MG1655 Mat fimbria variant. The matBCDEF genes from MG1655 were cloned under the inducible trc promoter in pSE380. When the resulting plasmid pMAT20 was transformed into MG1655, we observed Mat fimbriae expression (Fig. 2d) and biofilm formation (Fig. 2c) equivalent to that detected in the strain harbouring pMAT6. The same result was obtained when the two plasmids were expressed separately in the IHE 3034-Rif or the IHE 3034 matBCDEF background (data not shown). The results show that there is no critical impact of the allelic variations on the function of the encoded proteins. Furthermore, the results illustrate that the mat genes are functional in MG1655, thus suggesting that the repression of Mat fimbriae expression in MG1655 is a consequence of differences in regulatory mechanisms, similar to the silencing of the highly conserved curli operons (Römling et al., 1998) in a large number of E. coli strains (Hammar et al., 1995; Olsén et al., 1993; Uhlich et al., 2001). Interestingly, despite the induced Mat expression, no clear biofilm formation was detected at 37°C, indicating that under our test conditions the...
expression of Mat fimbriae is not enough for biofilm formation at 37 °C. Our results demonstrate that the presence of an expressed mat gene cluster is correlated with the ability of E. coli to produce a temperature-dependent biofilm, and we hypothesize that the lack of Mat-fimbriae-dependent biofilm at 37 °C results from downregulation of other surface structures (White-Ziegler et al., 2008) interacting with the Mat fimbriae. These factors remain to be identified.

**Flagella and other fimbriae of IHE 3034 are not critical for biofilm formation at low temperature**

Strain IHE 3034 expresses the mannoside-binding type 1 fimbriae, the sialic acid-binding S-fimbriae and flagella (Selander et al., 1986) in addition to Mat fimbriae. Since various E. coli surface structures participate in biofilm formation (Van Houdt & Michiels, 2005), we compared surface filaments for their impact on biofilm formation by IHE 3034-Sm. No change in biofilm formation was observed by the single type 1 fimbrial fimA, S-fimbrial sfaA or flagellar fliC mutants, or with the fimA sfaA fliC triple mutant. However, the fimA sfaA fliC matB derivative, also deficient in Mat fimbriae production, failed to form a biofilm (Fig. 3), indicating that under these test conditions Mat fimbriae are a principal mediator of biofilm formation by IHE 3034.

![Fig. 3. Biofilm formation by IHE 3034 surface filament mutants.](image)

**Mat fimbriae affect early stages of biofilm development and are present in mature biofilms**

Biofilm formation initiates as surface attachment and then proceeds to a complex architecture of cell interactions (Stoodley et al., 2002). We next determined the initial attachment of IHE 3034-Rif derivatives onto a PVC surface, first by using the CV-staining method with 1 h incubation time at 20 °C. At low bacterial density, both the wild-type and the matBCDEF mutant adhered weakly and no difference was seen between the strains (Fig. 4a), whereas the adherence at higher bacterial density enabled the wild-type IHE 3034-Rif to bind efficiently on the PVC surface. Similar results were obtained by indirect immunofluorescence microscopy analysis of PVC tabs incubated with low-density bacterial suspensions. At early time points (≤1 h), the attachment of the matBCDEF mutant was indistinguishable from that of the wild-type strain (Fig. 4b). However, after 3 h, a difference between the wild-type and mutant became evident. Over time, IHE 3034-Rif formed a progressively denser layer of cells on the PVC surface, whereas the attachment of the matBCDEF mutant remained unchanged, indicating that the mutant was defective in irreversibly attachable and thus biofilm formation.

Expression of Mat fimbriae is evidently regulated by phase variation (Pouttu et al., 2001), an adaptive strategy to switch on and off the expression, leading to cell-to-cell variability in a population. The majority of the IHE 3034-Rif cells cultured statically in M63 medium and used to inoculate the wells with PVC tabs were Mat-fimbriated (69.6 ± 2.0 %), and no accumulation of Mat-positive cells was observed during the initial attachment experiment (Fig. 4b). Interestingly, these frequencies of Mat fimbriae-expressing cells were markedly higher than the frequencies observed earlier with IHE 3034-Rif cells cultured in LB (Pouttu et al., 2001), suggesting that the adjustment of the balance between the two fimbriation states responds to environmental signals. This may be responsible for the ~50 % lower level of biofilm formation when M63 was replaced with LB as a growth medium (data not shown).

Formation of a biofilm involves coordinate multifactorial reprogramming of gene expression, including induction or downregulation of fimbriae genes (Domka et al., 2007; Hancock & Klemm, 2007; Schembri et al., 2003). We next assessed whether Mat fimbriae are differentially expressed in sessile biofilm cells of IHE 3034-Rif and their planktonic counterparts. The cells from a glass flask, a substrate that does not support biofilm formation by IHE 3034-Rif, were used as a reference, as an inoculum, and were defined as ‘inoculate’ cells; the cells in the glass flasks at the end of the incubation periods were termed ‘planktonic’ cells. The non-attached cells removed from the PVC wells after biofilm growth were defined as ‘surface-influenced planktonic (SIP)’ cells, according to Steyn et al. (2001), and the substrate-attached cells harvested from the walls of the PVC wells were termed ‘biofilm’ cells (see Methods). After 24 or
48 h of static growth in M63 medium at 20 °C, the mean Mat expression levels of the entire cell populations were quantified by whole-cell ELISA. No changes in the surface expression of Mat fimbriae were apparent on IHE 3034-Rif cells harvested from the different modes of growth (Fig. 5a). We also analysed the cell samples by immunoblotting with anti-Mat antibodies to detect both surface expressed and intracellular fimbrial proteins. Consistent with the ELISA results, the 18 kDa band of the major fimbrillin MatB, corresponding to the calculated molecular mass of mature MatB (Pouttu et al., 2001), was expressed in similar amounts in all IHE 3034-Rif samples (Fig. 5b). Similarly, examination of the IHE 3034-Rif cell populations by indirect immunofluorescence microscopy with anti-Mat fimbriae antiserum was in line with these and the initial

**Fig. 4.** Initial attachment and Mat fimbriae expression of IHE 3034-Rif derivatives onto PVC surfaces. (a) Quantification of initial attachment in the PVC microtitre plate wells by CV staining. Bacteria were adjusted in M63 medium to low or high density (OD600 0.1 or 1.0, respectively) and incubated in the wells for 1 h at 20 °C. The data represent means and standard deviations of three independent experiments. (b) Indirect immunofluorescence microscopy analysis of the initial biofilm formation using primary antibodies directed against Mat fimbriae. The bacteria were adjusted in M63 medium to low density (OD600 0.1) and incubated at 20 °C in microtitre plates containing PVC tabs. At the indicated time points, tabs were removed and cells were fixed, immunostained and examined by epifluorescence microscopy. Representative microscopy fields of phase-contrast (left panels) and epifluorescence images (right panels) are shown, and the mean number of attached bacteria (left panels) and the proportion of Mat fimbriated bacteria (right panels) ± SD in 20 and 6 randomly chosen fields of 10^3 μm^2, respectively, is indicated below the images. Arrows indicate adherent, Mat fimbriated bacteria. Scale bar, 5 μm.

**Fig. 5.** Biofilm formation and Mat fimbriae expression by IHE 3034-Rif and the matBCDEF deletion mutant. Expression of Mat fimbriae in planktonic, surface-influenced planktonic (SIP) and biofilm cells of IHE 3034-Rif was analysed by whole-cell ELISA (a) and Western blotting of HCl-treated cellular proteins (b) with anti-Mat fimbria serum as primary antibodies after growth for 24 h or for 48 h in M63 medium at 20 °C. The whole-cell ELISA data represent means and standard deviations of three independent experiments. Western blotting was assessed twice, and a representative blot is shown.
attachment results, as 74 to 83% of the cells in the populations expressed the fimbriae (data not shown).

Conclusion

The *E. coli* strains IHE 3034 and ABU38 differ in Mat fimbriae expression and form biofilms that vary in temperature sensitivity. This is a further indication that *E. coli* isolates use multiple mechanisms for biofilm formation (Beloin et al., 2008; Van Houdt & Michiels, 2005). We (Pouttu et al., 2001) and others (Rendón et al., 2007) have found that Mat fimbriae represent the most common fimbrial type in *E. coli* and that the *mat* genes in *E. coli* of different origin are highly conserved (97 to 98% nucleotide sequence identity). This suggests that the Mat fimbria represents an ancestral fimbrial type in *E. coli* and that its functions are essential for *E. coli* as a species, although still poorly described. The current data indicate that the regulation of Mat fimbriae in different clonal groups of *E. coli* is variable. Enterohaemorrhagic, enterotoxigenic and enteropathogenic *E. coli* strains were shown to produce the fimbriae after growth at 37 °C in tissue culture medium, suggesting that intestinal *E. coli* strains may express the fimbriae in the human intestine (Blackburn et al., 2009; Rendón et al., 2007; Saldaña et al., 2009). Our ongoing studies indicate that IHE 3034 also is able to express the fimbriae at 37 °C, albeit under differential growth conditions (T. A. Lehti, unpublished). The central mechanism for Mat fimbriae expression in IHE 3034 is currently under investigation. Recently, Lasaro et al. (2009) reported that the Mat fimbria homologue of the probiotic *E. coli* strain Nissle 1917 is essential for intestinal colonization of the infant mouse. In the mucosal habitats of *E. coli*, multispecies biofilm communities are present on the gut (Palestrant et al., 2004) and vaginal (Swidsinski et al., 2005) epithelia and there is evidence for the in vivo community behaviour of commensal and pathogenic *E. coli* (Macfarlane & Macfarlane, 2006; Rosen et al., 2007), suggesting that the structured lifestyle contributes both to normal ecology and to pathogenicity of *E. coli* in the host. Furthermore, the ability of *E. coli* to grow protective biofilms on non-host targets in heterogeneous environmental niches arguably ensures continual transmission to human populations. The results presented in this study show that the expression of the ubiquitous *mat* fimbrial operon enables *E. coli* to display multicellular behaviour at low temperatures. It remains to be established whether Mat-mediated biofilm formation plays a role in environmental survival and/or in host colonization by *E. coli*.

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