Regulation of type 1 fimbriae synthesis and biofilm formation by the transcriptional regulator LrhA of *Escherichia coli*

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Type 1 fimbriae of *Escherichia coli* facilitate attachment to the host mucosa and promote biofilm formation on abiotic surfaces. The transcriptional regulator LrhA, which is known as a repressor of flagellar, motility and chemotaxis genes, regulates biofilm formation and expression of type 1 fimbriae. Whole-genome expression profiling revealed that inactivation of *lrhA* results in an increased expression of structural components of type 1 fimbriae. *In vitro*, LrhA bound to the promoter regions of the two *fim* recombinases (FimB and FimE) that catalyse the inversion of the *fimA* promoter, and to the invertible element itself. Translational *lacZ* fusions with these genes and quantification of *fimE* transcript levels by real-time PCR showed that LrhA influences type 1 fimbrial phase variation, primarily via activation of FimE, which is required for the ON-to-OFF transition of the *fim* switch. Enhanced type 1 fimbrial expression as a result of *lrhA* disruption was confirmed by mannose-sensitive agglutination of yeast cells. Biofilm formation was stimulated by *lrhA* inactivation and completely suppressed upon LrhA overproduction. The effects of LrhA on biofilm formation were exerted via the changed levels of surface molecules, most probably both flagella and type 1 fimbriae. Together, the data show a role for LrhA as a repressor of type 1 fimbrial expression, and thus as a regulator of the initial stages of biofilm development and, presumably, bacterial adherence to epithelial host cells also.

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INTRODUCTION

The *lrhA* gene of *Escherichia coli* encodes a transcriptional regulator of the LysR family (Bongaerts *et al.*, 1995). Recently, the function of LrhA has been elucidated by a microarray-based comparison of the transcriptional profiles of wild-type *E. coli* and an isogenic *lrhA* mutant. LrhA has been identified as a regulator of genes involved in flagellation, motility and chemotaxis (Lehnen *et al.*, 2002). The *lrhA* mutants showed an increased expression of flagellar, motility and chemotaxis genes (e.g. *flhDC*, *fliA*, *fliC*) and a

corresponding increased motility and chemotactic response. LrhA acts as transcriptional repressor of the *flhDC* genes encoding the FlhD₂C₂ master regulator, and thus of the *flhDC* regulon. The *flhDC* regulon contains nearly 50 genes required for flagellum biosynthesis and function, which are arranged in at least 13 operons and whose expression is under hierarchical control (Kalir *et al.*, 2001; Macnab, 1996). LrhA also controls its own expression, but unlike most LysR-type regulators, LrhA is positively autoregulated (Lehnen *et al.*, 2002). In addition, the LrhA protein also controls the stationary-phase sigma factor σ^S (RpoS), which regulates gene expression in response to general stress in enteric bacteria (Gibson & Silhavy, 1999; Mukherjee *et al.*, 2000). The concentration of σ^S is regulated through degradation by the ClpXP protease. LrhA represses RpoS stability by affecting the function of ClpXP and the response regulator RssB (or SprE), which serves as a σ^S recognition factor.

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Abbreviations: Ap, ampicillin; H-NS, histone-like nucleoid-structuring protein; IHF, integration host factor; IRL, left inverted repeat; Km, kanamycin; Lrp, leucine-responsive regulatory protein; Sm, spectinomycin; UPEC, uropathogenic *E. coli*.

Differential gene-expression studies have indicated that LrhA also represses structural (*fimAICDFG*) and regulatory (*fimE*) genes related to the synthesis of type 1 fimbriae (Lehnen *et al.*, 2002). Type 1 fimbriae of *E. coli* mediate adherence to mannose-containing receptors, which is an important early step in colonization of host cells (Brinton, 1959; Keith *et al.*, 1986; Mulvey *et al.*, 1998). The fimbriae consist of repeating structural subunits (FimA) and several minor components, including an adhesin (FimH) (Jones *et al.*, 1995). The expression and assembly of type 1 fimbriae require at least eight genes that are localized within one gene cluster. The expression of the *fim* structural subunit genes (*fimA–H* operon) is controlled by an invertible 314 bp promoter element ('*fim* switch'). Inversion of the 314 bp switch element is the basis of phase-variable expression of type 1 fimbriae, which leads to a heterogeneous population

of fimbriate (phase-ON) and afimbriate (phase-OFF) cells. The *fim* switch is promoted by the recombinases FimE and FimB. As a result, the *fimA* promoter can be in the OFF orientation (no transcription) or in the ON orientation, which allows transcription (Klemm, 1986). FimB facilitates inversion from phase-OFF to phase-ON, as well as inversion from phase-ON to phase-OFF. FimE, on the other hand, causes only switching from phase-ON to phase-OFF (Blomfield *et al.*, 1991; Gally *et al.*, 1996). In addition to the *fim* recombinases, efficient inversion of the *fim* switch requires the accessory proteins integration host factor (IHF) and leucine-responsive regulatory protein (Lrp) (Blomfield *et al.*, 1993, 1997; Dorman & Higgins, 1987; Gally *et al.*, 1994). The histone-like nucleoid-structuring protein (H-NS) is also involved in the recombination event (Donato *et al.*, 1997; O'Gara and Dorman, 2000; Olsen & Klemm, 1994).

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

Strain/plasmid/phage	Genotype and characteristics	Reference or source
Strains		
MG1655	Wild-type <i>E. coli</i> K-12	Jensen (1993)
MC4100	F [−] <i>araD</i> 139 Δ(<i>argF-lac</i>)U169 <i>rpsL</i> 150 <i>relA</i> 1 <i>flhD</i> 530 <i>deoC</i> 1 <i>ptsF</i> 25 <i>rbsR</i>	Silhavy <i>et al.</i> (1984)
LJ10	MG1655, but Δ(<i>argF-lac</i>)U169, <i>zdh</i> -Tn10 (Tet ^R)	K. Jahreis, Osnabrück
JM105	<i>thi</i> <i>strA</i> <i>endA</i> <i>sbcB</i> 15 <i>hsdR</i> 4 (<i>lac-proAB</i>) F'(<i>traD</i> 36 <i>proAB</i> ⁺ <i>lac</i> ^f <i>lacZ</i> ΔM15)	Yanisch-Perron <i>et al.</i> (1985)
AAEC185	YMC9 Δ <i>fim</i> operon Δ <i>lac</i> <i>recA</i> <i>endA</i> 5	Blomfield <i>et al.</i> (1991)
536	Uropathogenic wild-type <i>E. coli</i> (O6:K15:H31), Sm ^R	Berger <i>et al.</i> (1982)
IMW41	MC4100, but <i>lrhA</i> :: <i>spc</i> ^R	Bongaerts <i>et al.</i> (1995)
IMW325	MG1655, but <i>lrhA</i> :: <i>spc</i> ^R	This work
IMW330	<i>E. coli</i> 536, but <i>lrhA</i> :: <i>kan</i> ^R	This work
IMW337	LJ10, but λ(Φ(<i>fimE'</i> −' <i>lacZ</i>))hyb	This work
IMW338	LJ10, but <i>lrhA</i> :: <i>spc</i> ^R λ(Φ(<i>fimE'</i> −' <i>lacZ</i>))hyb	This work
IMW339	LJ10, but λ(Φ(<i>fimA'</i> −' <i>lacZ</i>))hyb	This work
IMW340	LJ10, but <i>lrhA</i> :: <i>spc</i> ^R λ(Φ(<i>fimA'</i> −' <i>lacZ</i>))hyb	This work
IMW414	LJ10, but λ(Φ(<i>fimB'</i> −' <i>lacZ</i>))hyb	This work
IMW415	LJ10, but <i>lrhA</i> :: <i>spc</i> ^R λ(Φ(<i>fimB'</i> −' <i>lacZ</i>))hyb	This work
IMW461	MC4100, but λ(Φ(<i>fimE'</i> −' <i>lacZ</i>))hyb	This work
IMW462	IMW41, but λ(Φ(<i>fimE'</i> −' <i>lacZ</i>))hyb	This work
SM10λpir	<i>thi</i> 1 <i>thr</i> 1 <i>leu</i> B6 <i>supE</i> 44 <i>tonA</i> 21 <i>lacY</i> 1 <i>recA</i> [−] ::RP4-2-Tc::Mu Km ^R	Miller & Mekalanos (1988); Simon <i>et al.</i> (1983)
SY327λpir	F [−] <i>araD</i> Δ(<i>lac pro</i>) <i>argE</i> (Am) <i>recA</i> 56 <i>rif</i> ^R <i>nalA</i> λpir	Miller & Mekalanos (1988)
Plasmids		
pCVD442	Suicide vector for gene inactivation (<i>sacB</i> ori ColE1 Amp ^R)	Donnenberg & Kaper (1991)
pGS607	pUC118 with 1.3 kb kanamycin resistance GenBlock fragment (Pharmacia)	Six <i>et al.</i> (1994)
pJL28	p('' <i>lacZ</i> , <i>bla</i> ⁺), protein fusion vector	E. Bremer, Marburg
pJL30	p('' <i>lacZ</i> , <i>bla</i> ⁺), protein fusion vector	E. Bremer, Marburg
pMW132	pET28a, but with complete <i>lrhA</i> gene for LrhA expression	Lehnen <i>et al.</i> (2002)
pMW192	pCVD442, but <i>lrhA</i> :: <i>kan</i> ^R	This work
pMW193	pJL28 derivative carrying the <i>fimE</i> promoter (nt −463 to +79)	This work
pMW194	pJL30 derivative carrying the <i>fimA</i> promoter (nt −532 to +22)	This work
pMW213	pME6010, but <i>lrhA</i> and promoter (−359 to end of <i>lrhA</i> gene)	Lehnen <i>et al.</i> (2002)
pMW251	pJL28 derivative carrying the <i>fimB</i> promoter (nt −1119 to +68)	This work
pMW282	pJL30 derivative carrying the <i>fimA</i> promoter locked ON (nt −400 to +22)	This work
Phage		
λRZ5	λ ' <i>bla</i> , ' <i>lacZ</i> , <i>lacY</i> ⁺	Ostrow <i>et al.</i> (1986)

Fimbriae-mediated adherence is important for the virulence of uropathogenic *E. coli* (UPEC), and the expression of type 1 fimbriae is linked to urinary tract colonization and pathogenesis (Connell *et al.*, 1996; Martinez *et al.*, 2000; Mulvey, 2002). Type 1 fimbriae have also been described as key factors in biofilm establishment on abiotic surfaces (Pratt & Kolter, 1998; Schembri & Klemm, 2001). Under static growth conditions, type 1 fimbriae provide stable interactions between bacteria and different surfaces, including polystyrene, PVC, polycarbonate and borosilicate glass – a prerequisite for biofilm formation. The non-specific attachment to abiotic surfaces and the specific binding to receptor molecules are mannose-inhibitable and are mediated by the fimbrial adhesin FimH. Thus, in addition to other specific outer-membrane proteins, type 1 fimbriae promote the transition from a free-living, planktonic existence to a complex sessile lifestyle.

The aim of this study was to examine the role of LrhA in the regulation of type 1 fimbriae expression at the molecular level and with regard to adherence and biofilm formation.

METHODS

Bacterial strains, plasmids and media. The bacterial strains, plasmids and phage used are shown in Table 1. Unless otherwise stated, plasmids were maintained in *E. coli* JM105 (Yanisch-Perron *et al.*, 1985). For genetic experiments, the bacteria were routinely grown in Luria-Bertani (LB) broth and on LB agar. For expression studies, M9 medium (Miller, 1992) supplemented with acid-hydrolysed Casamino acids (0.1%), tryptophan (0.005%) and 20 mM glycerol was used. Cultures were incubated aerobically at 37 °C in flasks with shaking at 180 r.p.m. When required, antibiotics were added as follows: 100 µg ml⁻¹ ampicillin (Ap), 50 µg ml⁻¹ kanamycin (Km), 50 µg ml⁻¹ spectinomycin (Sm), and 20 µg ml⁻¹ tetracycline. Cell densities were measured as the OD at 578 nm.

RNA preparation. RNA was extracted from bacteria grown aerobically at 37 °C in the modified M9 mineral medium. For DNA array analysis, total RNA was extracted as described by Lehen *et al.* (2002) and Wendisch *et al.* (2001) from cells harvested at OD₅₇₈ 0.3–0.45. For real-time RT-PCR, total RNA was extracted from cultures grown to an OD₅₇₈ ~0.5 using RNeasyProtect Bacteria Mini kits (Qiagen) as recommended by the manufacturer. Traces of genomic DNA were removed by DNase treatment with DNA-free reagent (Ambion).

DNA microarray and macroarray ('*E. coli* Pathoarray') analysis. Microarray preparation, probe labelling, hybridization and analysis were carried out as described previously (Lehen *et al.*, 2002; Wendisch *et al.*, 2001). The DNA macroarray covered 456 pathogenicity island- or virulence-associated genes of UPEC strain 536 and other extraintestinal and intestinal pathogenic *E. coli* (including *Shigella*) and has been described previously (Dobrindt *et al.*, 2002, 2003). In order to compare the gene expression of wild-type strain 536 and its isogenic *lrhA* mutant, '*E. coli* Pathoarrays' were hybridized in five different experiments using independently labelled cDNA probes. Reverse transcription of RNA, radioactive probe labelling and hybridization were performed as described by Dobrindt *et al.* (2003). The scanned arrays were analysed with ArrayVision software (Imaging Research, St. Catharines, Canada) followed by visual inspection. For identification of genes with statistically significant changes in expression, significance analysis of microarrays

(SAM), a statistical technique for finding significant genes in a set of array experiments, was used (Tusher *et al.*, 2001) (<http://www-stat.stanford.edu/~tibs/SAM/>).

Determination of *fim* transcript levels by quantitative, real-time PCR following reverse transcription. Before reverse transcription reactions were performed, the absence of DNA from RNA samples was verified by PCR amplification (40 cycles) of the *fimE*, *fimA*, *fimI* and *gapA* genes with 0.2 µg RNA as template. cDNA synthesis was performed with 320–550 ng total RNA, isolated from three independent bacterial cultures, and random hexamer primers, using the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions.

The resulting cDNA samples with concentrations varying between 1.7 and 2.5 µg µl⁻¹ were diluted 100- to 1000-fold prior to real-time PCR. PCR reactions were done using the iQ SYBR Green Supermix (Bio-Rad) and the following specific primers (Table 2): FimE_for and FimE_rev for amplification of *fimE*, FimA_for and FimA_rev for amplification of *fimA*, and FimI_for and FimI_rev for amplification of *fimI*. As an LrhA-independent internal control, *gapA* (encoding D-glyceraldehyde-3-phosphate dehydrogenase A) was amplified using primers GapA_for and GapA_rev. The reactions containing 1 × iQ SYBR Green Supermix, 100 nM of each primer and 5 µl of the template cDNAs were run on an iCycler iQ system (Bio-Rad) with the following cycling parameters: initial denaturation at 95 °C for 3 min and 45 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 30 s. Each PCR was performed in duplicate. Specificity for all amplicons was confirmed via melting curves and gel analysis.

The threshold cycle for each real-time PCR was determined by using the software package supplied with the iCycler system, and *fimA*, *fimE* and *fimI* expression was normalized with *gapA* expression for each sample.

DNA manipulation and cloning procedures. DNA cloning and preparation of plasmid and chromosomal DNA were performed according to standard methods (Sambrook *et al.*, 1989). The *fimE*–'*lacZ*' fusion plasmid (pMW193) was constructed by PCR amplification of the promoter region of *fimE* (–463 to +79) from genomic

Table 2. Primers used in this study

Primer	Sequence (5'→3')
FimE_for	TAT GAA TTG GCG GAG CGT GGT G
FimE_rev	AAA CGA GCA GCA TTA CTG GCG GTA T
FimA_for	GGA CAG GTT CGT ACC GCA TC
FimA_rev	ACG TTG GTA TGA CCC GCA TC
FimI_for	GAC GGT CAA TAT GGG GCA AA
FimI_rev	TTT TTA CCA TCC GCG ACA CC
GapA_for	GTT GTC GCT GAA GCA ACT GG
GapA_rev	AGC GTT GGA AAC GAT GTC CT
<i>fimE</i> HindIII	AAG ACA ATA AGC TTT GGC TCC CG
<i>fimE</i> BamHI	AGA TAG TTG GGA TCC CAG GGA T
<i>fimB</i> EcoRI	CCT TTC AGA CTG AAT TCC GTT TTC ATC
<i>fimB</i> HindIII	CCG GTA TTT GAA GCT TTA AGG AGT G
<i>fimA</i> BamHI	GTT TTG CCG GAT CCT GGG AAA
<i>fimA</i> HindIII	CGA CAG AAC AAA GCT TGC CAG AG
<i>fimA2</i> BamHI	CAT AGA GGA AAG GAT CCC GGA C
<i>lrhA</i> BglII	GGA CCA CAG ATC TCA TAA CAG A
<i>lrhA3</i>	GTC TCA GGA ATT CTC TAT CGT CCG

DNA of strain MG1655 with primers (Table 2) *fimEHindIII* and *fimEBamHI*. The resulting 542 bp fragment comprising the complete intergenic region was cloned into the *HindIII* and *BamHI* sites of the protein fusion vector pJL28. The *fimB'*-*'lacZ* (-1119 to +68; plasmid pMW251), *fimA'*-*'lacZ* (-532 to +22; plasmid pMW194) and *fimA2'*-*'lacZ* (-400 to +22; plasmid pMW282) fusions were constructed in the same way with the following primers (Table 2): *fimBEcoRI* and *fimBHindIII*, *fimABamHI* and *fimAHindIII*, and *fimA2BamHI* and *fimAHindIII*, respectively. After sequence verification, translational *lacZ* fusions were transferred to the genome of *E. coli* (wild-type and *lrhA* mutant) with phage λ RZ5 (Ostrow *et al.*, 1986), and monolysogens were identified and used for further work (Bongaerts *et al.*, 1995). Fusion *fimA2'*-*'lacZ* on plasmid pMW282 was introduced into receptor strains by chemical transformation. The construction of plasmid pMW132 used for the overexpression and isolation of His₆-LrhA has been described previously (Lehnen *et al.*, 2002).

Inactivation of the *lrhA* gene in *E. coli* strain 536 by allelic exchange. The *lrhA* gene was inactivated by insertion of an Ω -Km cassette in the unique *StuI* site and cloned into pCVD442, a suicide vector that confers sensitivity to sucrose in Gram-negative bacteria (Donnenberg & Kaper, 1991). First, the *lrhA* gene was amplified with flanking up- and downstream regions by PCR using primers *lrhABglII* and *lrhA3* (Table 2), and cloned in pBluescriptKS⁻ cut with *BamHI* and *EcoRI*. After insertion of the Ω -Km cassette originating from plasmid pGS607, a hybrid plasmid with pCVD442 was constructed by *SalI* restriction of both plasmids and subsequent ligation. By deletion of the *SacI* fragment, *bla* and the origin of pBluescriptKS⁻ were eliminated. The resulting plasmid (pMW192) allowed allelic exchange with the chromosomal *lrhA* gene of *E. coli* 536, resulting in strain IMW330. Vector pCVD442 and derivatives thereof, which carry a *pir*-dependent origin of replication, were propagated in the *E. coli* strain SY327 λ pir. Allelic exchange was performed as previously outlined (Nagy *et al.*, 2002). Briefly, plasmid pMW192 was mobilized from the donor strain *E. coli* SM10 λ pir into the recipient strain *E. coli* 536 (Sm^R). Isolates that were sucrose-resistant and Ap-sensitive, which is indicative of the loss of vector sequence, as well as Km- and Sm-resistant, were selected for further studies. The allelic exchange was confirmed by PCR amplification of the chromosomal region.

Gel retardation. For gel-retardation assays, DNA fragments containing *fimE* (570 bp), *fimB* (1218 bp) and *fimA* (554 bp) promoter regions were used. They were obtained by PCR amplification of chromosomal DNA from a colony of MG1655 with primers *fimEHindIII* and *fimEBamHI*, *fimBEcoRI* and *fimBHindIII*, and *fimABamHI* and *fimAHindIII*, respectively. After digestion with the appropriate restriction enzymes, the promoter fragments were purified and labelled with [α -³²P]dATP on both strands, as previously described (Lehnen *et al.*, 2002).

The gel-retardation assays were performed essentially as described by Drapal & Sawers (1995). The His₆-LrhA proteins were purified as described earlier (Lehnen *et al.*, 2002) and incubated with labelled DNA (5 nM) in binding buffer (10 mM Tris/HCl, pH 7.5, 10%, w/v, glycerol, 2.5 mM EDTA, 50 mM KCl, 0.1 mM DTT, 4 mM spermidine, 12.5 μ g sonicated calf thymus DNA, 1 μ g BSA, in a final volume of 20 μ l) for 30 min at room temperature. After incubation, the reaction mix was applied to a non-denaturing polyacrylamide gel (5%) buffered with Tris/borate/EDTA (Sambrook *et al.*, 1989). The concentration of LrhA required for half-maximal retardation was designated apparent *K*_D.

β -Galactosidase assay. The strains were grown at 37 °C in supplemented M9 medium with glycerol (20 mM). From exponentially growing bacteria (OD₅₇₈ 0.5–0.6), β -galactosidase specific activities were measured and calculated (Miller, 1992).

Agglutination of yeast cells, haemagglutination and haemolysis. The capacity of bacterial strains to express a D-mannose-binding phenotype was measured by the ability to agglutinate *Saccharomyces cerevisiae* cells on glass slides. Aliquots of washed bacterial suspensions of various optical densities were incubated with a yeast suspension (10 mg ml⁻¹ dry weight). Agglutination, which is susceptible to inhibition by D-mannose (2%), was monitored visually by aggregation and precipitation of the cells. The lowest OD₅₇₈ of bacterial suspensions at which agglutination was observed was determined.

The presence of P and S fimbriae was demonstrated by haemagglutination of defibrinated human and bovine erythrocytes, respectively. A suspension of human and bovine blood (Elocin-lab, Muelheim) was mixed on a glass slide with a toothpicked colony of *E. coli*. After incubation for some minutes on ice, agglutination occurred. Expression of S or P fimbriae was also checked by Western blot analysis using polyclonal sera raised against S or P fimbriae of *E. coli* strain 536, as described by Nagy *et al.* (2002).

To test their haemolytic activity, cells from *E. coli* colonies were spread onto sheep blood agar plates (Oxoid) with a toothpick and incubated overnight at 37 °C. Lysis of the blood cells by α -haemolysin was detected by formation of clear haloes around the colonies after incubation. Haemolytic activities of culture supernatants were determined quantitatively using a method previously described (Nagy *et al.*, 2002).

Ascending urinary tract infection model. Intravesical infections of 3–4 day old CFLP mice (Gödöllő, Hungary) were performed as previously described (Allison *et al.*, 1994). Six to 26 infant mice were infected with each of the strains in two separate experiments. UPEC strain 536 and the *lrhA* mutant IMW330 were administered intravesically using an inoculum of 2.5×10^4 or 2.5×10^5 cells. The mortality rate was determined after 3 days of infection.

Biofilm formation. Overnight cultures were diluted 1:50 into LB broth and 200 μ l aliquots were transferred to the wells of a 96-well polystyrene microtitre plate (Nunc). For growth in the presence of 1% methyl- α -D-mannopyranoside (α MM), 20 μ l of a 10% α MM solution was added to 180 μ l LB broth inoculated with an overnight culture already grown in the presence of 1% α MM. After growth of the samples for 24 h at 30 °C, biofilm formation was measured. Medium and unbound cells were discarded, the wells were rinsed with water, and adhered cells were stained by incubation with 200 μ l 1% crystal violet (Merck) for 15 min. Non-bound dye was removed by thorough rinsing. Biofilm formation was subsequently quantified by adding 200 μ l ethanol (99%) twice to the stained microtitre wells. The ethanol was brought up to a volume of 1 ml (with water) and the absorbance of crystal violet was measured at 540 nm (O'Toole & Kolter, 1998).

Electron microscopy of flagella. Cells were washed with 50 mM potassium phosphate (pH 7.0) and fixed with glutaraldehyde (2%, v/v, final concentration). The cells were allowed to sediment to carbon-coated grids for 1–2 min. After negative staining with 1% uranyl acetate, the grids were examined with a Philips CM12 transmission electron microscope (Scharf *et al.*, 2001).

RESULTS

Transcriptional regulation of *fim* genes by LrhA

Preliminary experiments suggested an effect of LrhA on the mRNA levels of *E. coli* fimbrial genes. For a more detailed analysis, the expression profile of wild-type *E. coli* MG1655 genes was compared to that of the isogenic *lrhA* mutant

Table 3. Fimbrial genes with increased mRNA abundances in the *lrhA* mutant

mRNA from *E. coli* wild-type (MG1655) and the *lrhA*⁻ derivative IMW325 was used for DNA microarray analysis (Lehnen *et al.*, 2002). The relative mRNA levels are the mean from three different experiments and genes with relative mRNA levels increased by factors ≥ 1.6 are shown. All genes are members of the same operon and are transcribed in the order shown. An asterisk indicates *P* values of < 0.05 . The *P* values were calculated based on a *t* test distribution (Lehnen *et al.*, 2002). The function of the gene products is given according to http://coli.berkeley.edu/cgi-bin/ecoli/coli_entry.pl.

Gene	Function	Relative RNA levels in the <i>lrhA</i> mutant (-fold)
<i>fimA</i>	Major type 1 subunit fimbrin	3.3*
<i>fimI</i>	Structural component, surface structure	7.3*
<i>fimC</i>	Periplasmic chaperone	4.1*
<i>fimD</i>	Outer-membrane protein; export and assembly of type 1 fimbriae	ND
<i>fimF</i>	Structural component	1.6*
<i>fimG</i>	Structural component	4.7
<i>fimH</i>	Minor fimbrial component	2.8*

IMW325 by DNA microarray analysis after growth in mineral medium with glycerol. In addition to the genes involved in flagellation, motility and chemotaxis (Lehnen *et al.*, 2002), genes encoding type 1 fimbriae showed increased relative mRNA abundances in the *lrhA* mutant in three different experiments (Table 3). The identified genes belong to the *fimAICDFGH* operon, and the mRNA contents were increased by factors between 1.6 and 7.3. The microarray experiments suggested that LrhA is a negative transcriptional regulator of type 1 fimbrial genes. Expression

of the *fimAICDFGH* operon is regulated by the *fim* switch, which is accomplished by the recombinases FimE and FimB. The relative mRNA level of *fimE*, encoding FimE (ON-to-OFF switch), was elevated in the *lrhA* mutant by a factor of 6.9, but only two interpretable microarray experiments were obtained. For FimB, no information on the mRNA levels was obtained due to interference from high background fluorescence levels.

To determine the role of the resolvases FimB and FimE in the LrhA control of type 1 fimbrial gene expression, translational *lacZ* fusions of the *fimA*, *fimB* and *fimE* genes were constructed and inserted in single copies into the chromosome of wild-type and *lrhA* mutant strains. The β -galactosidase activities of the *fimA'*-*lacZ* fusion showed a large variation in different experiments, both in the wild-type and the *lrhA* background, presumably due to effects of promoter inversion (Table 4). Multiple repeated experiments suggested, however, that *fimA* expression increases by a factor of about two in the *lrhA*-negative background. The *fimA2'*-*lacZ* fusion lacks the left inverted repeat (IRL) essential for inversion of the *fimA* promoter region. Therefore, the switch is locked in the phase-ON orientation and any expression changes should reflect only transcription initiation levels at the *fimA* promoter. In contrast to the 'unlocked' *fimA'*-*lacZ* fusion, wild-type and *lrhA* mutant showed no difference in *fimA2'*-*lacZ* expression. Similarly *fimB'*-*lacZ* expression was not affected by LrhA, whereas the expression of *fimE'*-*lacZ* was decreased about 2.5-fold in the *lrhA* mutant compared to the wild-type (Table 4). Plasmid-borne *lrhA* expression complemented the *lrhA* mutant for *fimE'*-*lacZ* expression, which then reached the same level as that of the wild-type. Expression of *lrhA* from the plasmid had no significant effect on *fimB'*-*lacZ* expression. These results imply that LrhA has no direct effect on *fimA* promoter activity, but enhances *fimE* expression, thereby promoting the ON-to-OFF switch at *fimA*.

Table 4. Expression of translational *lacZ* fusions of type 1 fimbrial genes in wild-type and *lrhA* mutant strains

ND, Not determined.

Fusions*	Strains (wild-type/ <i>lrhA</i> ⁻)	β -Galactosidase activity (MU) [†]			Repression by LrhA (-fold)
		Wild-type	<i>lrhA</i> ⁻	<i>lrhA</i> ⁺ ‡	
<i>fimA'</i> - <i>lacZ</i>	IMW339/IMW340	935 $\pm$ 631	1948 $\pm$ 666	ND	2.1
<i>fimA2'</i> - <i>lacZ</i> §	LJ10 (pMW282) /IMW331 (pMW282)	10 000 $\pm$ 1600	10 600 $\pm$ 1950	ND	1.1
<i>fimE'</i> - <i>lacZ</i>	IMW337/IMW338	626 $\pm$ 261	252 $\pm$ 75	659 $\pm$ 179	0.4
<i>fimB'</i> - <i>lacZ</i>	IMW414/IMW415	140 $\pm$ 5	168 $\pm$ 6	155 $\pm$ 6	1.2

*Gene fusions inserted by λ RZ5 into the *att* site of the *E. coli* chromosome.

[†]Cells were grown under aerobic conditions in M9 medium supplemented with glycerol. The β -galactosidase activities (Miller Units, MU) are mean values of three or more independent experiments $\pm$ standard deviation.

[‡]Respective *lrhA* mutant complemented with plasmid-borne *lrhA* (pMW132).

§*fimA* promoter (-400 to +22) with deleted IRL site.

||*lacZ* fusion on plasmid.

The positive effect of LrhA on *fimE'*-*lacZ* expression is in conflict with the data obtained from the DNA microarray analysis, which show an increased *fimE* expression in the absence of LrhA (repression factor of 6.9 in two DNA-chip experiments compared to a factor of 0.40 in the *lacZ*-expression studies). To resolve this contradiction, the mRNA levels in wild-type and the *lrhA* mutant were determined by quantitative RT-PCR with RNA isolated from three independent cultures. The mRNA levels of *fimE* decreased by a factor of 2.9 in the *lrhA* mutant (Table 5), confirming the results obtained for the *fimE'*-*lacZ* reporter gene fusion rather than the results of the DNA microarray. The mRNA levels of *fimA* (1.7-fold) and *fimI* (1.9-fold), on the other hand, increased slightly in the *lrhA* mutant. The quantitative RT-PCR experiment of *fimA* (Table 5) was therefore in good agreement with the DNA microarray analysis and the *lacZ* reporter-gene studies.

The regulation of the flagella, motility and chemotaxis by LrhA is effected via the regulator FlhD₂C₂, which is under the transcriptional control of LrhA (Lehnen *et al.*, 2002). The requirement of FlhD₂C₂ for the LrhA-mediated transcriptional regulation of *fim* genes was tested in a *flhDC*-negative background. In a similar manner to the *flhDC*-positive background, inactivation of *lrhA* in mutant IMW462 resulted in a threefold decrease of *fimE'*-*lacZ* expression (545 ± 17 Miller units) compared to the parental strain IMW461 (170 ± 4 Miller units). Therefore, LrhA-steered control of *fim* gene expression is independent of FlhD₂C₂.

Binding of LrhA to the promoters of type 1 fimbrial genes

To demonstrate binding of LrhA to target promoters, gel-retardation experiments were performed. Promoter fragments of *fimA*, *fimE* and *fimB* were radioactively labelled and incubated with purified His₆-LrhA protein. When the samples were subjected to native gel electrophoresis, the band of free *fimA* promoter DNA disappeared at concentrations of 50–100 nM LrhA, and a DNA band with decreased electrophoretic mobility appeared. This band

presumably represents a DNA–LrhA complex and was formed with an apparent K_D of about 100 nM LrhA (Fig. 1A).

Similarly, gel retardation of *fimE* (Fig. 1B) and *fimB* promoter DNA by the addition of LrhA was observed. The concentrations of LrhA required for half-maximal retardation were 20–50 and 200–300 nM for the *fimE* and *fimB* promoter fragments, respectively. As for *fimA*, a large (625-fold) excess of non-specific DNA did not inhibit the formation of LrhA–promoter complexes, suggesting specific interactions between LrhA and the tested promoters. Thus, LrhA seems to bind to the promoter regions of all three genes, with the strongest binding affinity for the *fimE* promoter, but only *fimE* expression is directly influenced by LrhA.

Effect of *lrhA* mutation on agglutination of yeast, and biofilm formation

To verify an altered expression of the fimbrial genes in the *lrhA* mutant, various fimbriae-associated phenotypes and functions were tested in *E. coli* wild-type and *lrhA* mutants. Type 1 fimbriae of *E. coli* mediate adherence to mannose-containing receptors, allowing colonization of many host surfaces. The classical way to monitor type 1 fimbriae-mediated adhesion is agglutination of yeast (*S. cerevisiae*) cells. Agglutination was tested with the *E. coli* K-12 strains and the UPEC strain 536, since adhesion is of particular significance for pathogenic *E. coli* strains. The capacity of the wild-type strains and the corresponding *lrhA* mutants to agglutinate *S. cerevisiae* cells was tested on glass slides. Upon mixing, the agglutination reaction produced by the *lrhA* derivatives was significantly stronger and occurred at lower cell densities (OD_{578} 0.25) than with the wild-type (OD_{578} 1) (Table 6). Complementation of the *lrhA* mutant IMW325 with *lrhA* carried by plasmid pMW213 conferred a weak agglutination phenotype similar to that of the wild-type strains. As expected, the *fim* mutant strain AAEC185, lacking the fimbrial structural genes, caused no agglutination. Furthermore, the addition of mannose abolished agglutination completely. Thus, agglutination was specific for mannose-containing surface proteins and depended on the type 1 fimbriae, and LrhA repressed the agglutination phenotype.

Flagella, and type 1 and curli fimbriae, are the bacterial structures involved in the non-specific adhesion of *E. coli* to organic or inorganic surfaces, which represents the first step in biofilm formation (Pratt & Kolter, 1998; Vidal *et al.*, 1998). Attachment and adherence by fimbriae and flagella therefore represent important factors in biofilm formation. Since LrhA represses the synthesis of flagella (Lehnen *et al.*, 2002) and type 1 fimbriae, the effect of LrhA on biofilm formation under static growth conditions was examined. To this end, *E. coli* was grown in polystyrene microtitre plates for 24 h, and attached bacteria were visualized and subsequently quantified by staining with crystal violet (Fig. 2). The *lrhA* mutant IMW325 displayed a significantly

Table 5. Quantification of *fimA*, *fimE* and *fimI* gene expression in *E. coli* wild-type (MG1655) and *lrhA* mutant (IMW325) by quantitative RT-PCR

RNA was prepared from three independent cultures grown in M9 medium with glycerol to OD_{578} 0.5. Using primers specific for each of the indicated genes, RNA levels were quantified by RT-PCR relative to the level of the housekeeping gene *gapA*. The level of the genes in wild-type background was set at 1.

Gene	Function	Relative RNA levels
<i>fimE</i>	'Regulator' for <i>fimA</i> ; resolvase	0.34 ± 0.18
<i>fimA</i>	Major type 1 subunit fimbrin	1.67 ± 1.01
<i>fimI</i>	Structural component	1.87 ± 1.02

Fig. 1. Gel-retardation assay with a 554 bp *fimA* (A) and a 570 bp *fimE* (B) promoter DNA fragment and purified LrhA protein. The radioactively labelled fragment (5 nM in each lane) was incubated with various concentrations of LrhA in the presence of a 625-fold excess of competitor DNA and subjected to non-denaturing DNA PAGE (5% acrylamide). Arrows indicate bands of free DNA and of a retarded LrhA-DNA complex.

increased biofilm formation compared to the wild-type strain MG1655, which adhered to the well surface but to a lesser extent (Fig. 2A). When the *lrhA* mutant was complemented with a plasmid-borne *lrhA* gene, IMW325

Table 6. Effect of *lrhA* mutation on *E. coli* agglutination of yeast cells compared to wild-type and *fim*-negative *E. coli* strains with and without mannose

Cell suspensions of different optical densities were incubated with yeast cells and agglutination was detected. NA, no agglutination.

Strain (relevant genotype)	Agglutination of yeast (10 mg ml ⁻¹) beginning at OD ₅₇₈ :	
	-Mannose	+ 2% mannose
MG1655 (<i>E. coli</i> K-12 wild-type)	1.0	NA
IMW325 (MG1655, but <i>lrhA</i>)	0.25	NA
IMW325 (pMW132) (<i>lrhA</i> ⁺)	1.0	NA
AAEC185 (<i>fim</i> ⁻)	NA	NA
<i>E. coli</i> 536 (wild-type)	1.0	NA
IMW330 (<i>E. coli</i> 536, but <i>lrhA</i> ⁻)	0.25	NA

(pMW213), biofilm formation was no longer visible, presumably due to an increased *lrhA* copy number. No biofilm development was observed for the type 1 fimbriae-negative mutant strain AAEC185, demonstrating the requirement of type I fimbriation to build biofilms. Quantitative analysis of biofilm formation by measuring the dye adsorbed to the attached bacteria (O'Toole & Kolter, 1998) confirmed these phenotypes (Fig. 2B).

Next we tried to dissect the contribution of flagella and type 1 fimbriae to biofilm formation and the role therein of LrhA. *E. coli* MC4100, an *flhDC* deletion strain which lacks flagella but not fimbriae synthesis, showed largely decreased biofilm formation (Fig. 2C). In the *flhDC lrhA* double mutant strain IMW41, biofilm formation was impaired to a similarly low degree. The ability of type 1 fimbriae to bind both specifically to mannose and non-specifically to abiotic surfaces can be abolished by addition of methyl- α -D-mannopyranoside (α MM), a non-metabolizable analogue of mannose (Pratt & Kolter, 1998). This feature was exploited to further analyse the role of LrhA and type 1 fimbriae on biofilm formation. In the presence of α MM, biofilm formation was almost completely eliminated for both the wild-type and the *lrhA* mutant to similar levels to those of the *fim* deletion strain,

Fig. 2. Effects of *LrhA* on biofilm formation under static conditions. Cultures of MG1655, its isogenic *LrhA* mutant IMW325, the complemented strain IMW325 (pMW213) and the *fim*-negative strain AAEC185 were grown in polystyrene microtitre plates for 24 h. (A) Representative samples stained by crystal violet; (B) quantification of biofilm-associated dye, as described in Methods. In addition (C), the flagella-negative MC4100 (*flhDC*) and IMW330 (*flhDC* *LrhA*) strains were analysed for biofilm-associated dye. (D) Quantification of biofilm formation in the presence of 1% methyl- α -D-mannopyranoside (α MM). Experiments were done in triplicate. Error bars represent standard deviation.

which was not influenced by the addition of α MM (Fig. 2D). These results confirmed that under static growth conditions, both type 1 fimbriae and flagella are important for biofilm formation, and even suggested a slightly more prominent role for type 1 fimbriae. Since both flagella and type 1 fimbriae are essential for the development of biofilms, the clear effect of *LrhA* on biofilm formation has to be the result of the repression of both flagellar as well as type 1 fimbrial genes by *LrhA*.

Flagella in wild-type *E. coli* and *LrhA* mutants (electron microscopy)

The influence of *LrhA* on the number of flagella per cell was studied by electron microscopy of *E. coli* wild-type MG1655 and *LrhA* mutant IMW325. On average, the *LrhA* mutant showed an about threefold increased number of flagella compared to the wild-type (Fig. 3), including the number of attached and broken flagella. It was not possible

to quantify the difference in length of the flagella in electron micrographs due to frequent twisting and breakage of the flagella. Even so, these results indicate that the increased motility of an *LrhA* mutant (Lehnen *et al.*, 2002) can be attributed to an increased number of flagella per cell.

Significance of *LrhA* for virulence of UPEC

Type 1 fimbriae, together with other virulence factors, including several fimbrial adhesins, toxins and iron-uptake systems, contribute to the ability of UPEC to colonize the urinary tract. To test whether additional adhesins and other virulence factors specific for UPEC strains are also under the control of the *LrhA* protein, we used the '*E. coli* pathoarray', which carries 456 DNA probes for typical virulence-associated genes of extra-intestinal and intestinal pathogenic *E. coli*, and *Shigella* (Dobrindt *et al.*, 2003). The mRNA profile of UPEC strain 536 (O6:K15:H31), which

Fig. 3. Electron micrographs of wild-type *E. coli* MG1655 (A) and *LrhA* mutant strain IMW325 (B). The bacteria were grown overnight in LB broth, harvested by centrifugation, fixed and negatively stained with uranyl acetate.

expresses virulence factors such as S- and P-related fimbriae, α -haemolysin, yersiniabactin and capsule (Dobrindt *et al.*, 2003), was compared with that of its isogenic *LrhA* mutant IMW330. The genes encoding type 1-, S- and P-fimbrial determinants (*fim*, *sfa* and *prf* genes), α -haemolysin and ORF32 showed moderately increased mRNA levels (about twofold) in the *LrhA* mutant relative to the wild-type. Other virulence-related genes showed no significant alteration in transcript levels. Physiological tests for S and P fimbriae that measure their ability to bind to sialic-acid-specific receptors on bovine erythrocytes or Gal α [1-4]Gal β disaccharides on human erythrocytes showed no difference between the strains. Similarly, Western blot analysis with specific antibodies revealed no significant changes in the amount of S and P fimbriae (data not shown). The same applied to haemolytic activities and the overall virulence in an ascending urinary tract infection model in suckling mice, which gave no significant difference for wild-type and the *LrhA* mutant. It was concluded that type 1 fimbriae, but not other virulence factors, are transcriptionally regulated by LrhA.

DISCUSSION

It has previously been established that LrhA is a key repressor of genes associated with motility, chemotaxis and flagella synthesis in *E. coli* (Lehnen *et al.*, 2002). The present investigation demonstrates that LrhA serves also as a repressor of type 1 fimbriae expression. Due to its effects on flagella and fimbriae synthesis, LrhA is also a regulator of biofilm formation.

Control of fimbrial expression

Gene-expression profiling experiments revealed an upregulation of the genes belonging to the *fimAICDFGH* operon in the *LrhA* mutant. The expression of the structural genes for type 1 fimbriae is regulated by the site-specific inversion of the *fimA* promoter region by the two recombinases FimE and FimB. Although LrhA binding to the promoter regions of *fimA*, *fimB* and *fimE* was demonstrated, only *fimE* expression seemed to be directly influenced by LrhA. The expression and mRNA levels of *fimE* were decreased in the *LrhA* mutant compared to the wild-type, whereas mRNA

abundances of the structural genes for type 1 fimbriae were elevated in the *lrhA* mutant. These results led us to propose a model (Fig. 4) in which LrhA positively controls the expression of *fimE*, as demonstrated by *lacZ* fusion and quantitative RT-PCR. Since FimE exhibits a strong bias for phase switching from the ON to the OFF orientation, expression of the *fim* operon is reduced in the wild-type. In the absence of LrhA, however, *fimE* expression is decreased, leading to an elevated fimbrial expression in the *lrhA* mutant. This model is further supported by the fact that LrhA does not affect *fimA* promoter activity (in the locked-ON situation), even though LrhA binds to the *fimA* promoter. The binding of LrhA to the *fimE* promoter was specific, with an estimated apparent K_D value between 20 and 50 nM, whereas a 10-fold-higher LrhA concentration (200–300 nM) was required for half-maximal retardation of the *fimB* promoter. Other promoters proven to be targeted by LrhA, such as the *flhDC* and *lrhA* promoters, were bound by LrhA with a similar K_D to that of the *fimE* promoter (Lehnen *et al.*, 2002). These observations suggest that the binding of the *fimE* promoter by LrhA is more relevant for the regulation of fimbrial expression, and that the effect on *fimB* might be too small to be detected, if indeed it is present at all. Whether LrhA directly activates *fimE* expression, which is in contrast to the function of LrhA as a repressor for other target genes, or whether it alleviates the action of an additional repressor, remains to be elucidated.

The binding affinity of LrhA for the *fimA* promoter lies in-between those for the *fimE* and *fimB* promoters. Although our model predicts that LrhA controls expression of the *fim* genes primarily by controlling *fimE* expression, we cannot exclude that LrhA influences the *fim* switch by other mechanisms also; in other words, LrhA binding to the *fimA* promoter might influence site-specific inversion in a similar

manner to Lrp, IHF and H-NS (Blomfield *et al.*, 1993, 1997; O’Gara & Dorman, 2000). Lrp, for instance, slightly affects the transcription of both *fimB* and *fimE*. Additionally, it also binds with high affinity within the *fim* switch, and this binding stimulates recombination in both directions (Gally *et al.*, 1994). In the case of H-NS, it has been shown that H-NS is a repressor of the FimB recombinase, but there is evidence that this regulation is not the sole cause of the increased DNA flipping observed in *hns* mutant backgrounds (Donato *et al.*, 1997). By binding to the *fim* switch, LrhA might also compete with Lrp and/or IHF for DNA binding and disturb the structure needed for the recombination event. Alternatively, LrhA could steer the phase switching by directly influencing FimE or FimB activity. Such a mechanism has been shown for PapB, a transcriptional regulator of pyelonephritis-associated pili (*pap*) that has a negative effect on the expression of type 1 fimbriae. *In vitro* recombination assays have demonstrated that PapB directly inhibits FimB-promoted switching. Moreover, PapB promotes *fimE* expression to a similar degree to LrhA (Xia *et al.*, 2000).

The expression of *fimE* is also controlled by the orientation of the *fim* switch, which operates only when *fimE* is *in cis* to the invertible element (Sohanpal *et al.*, 2001). The expression of *fimE-lacZ* fusions is not subject to this negative control that is exhibited by the *fim* switch in the OFF orientation, whereas quantitative RT-PCR of *fimE* transcripts mirrors also the orientational control. Nevertheless, both approaches revealed a similar reduction of *fimE* expression in the *lrhA* mutant. These independent results are more reliable than the *fimE* expression data obtained by microarray analysis, which implied a regulation in the opposite direction. Transcript profiling with whole-genome microarrays is well suited to monitor the global changes associated with an altered genotype. However, alternative methods should be used to confirm single results.

Future experiments will have to determine whether the repression of type 1 fimbriae synthesis by LrhA can be solely attributed to the LrhA control of *fimE* expression or whether additional mechanisms influencing the phase variation play a role.

Biofilm formation

Biofilm development was strongly affected by LrhA, with increased biofilm formation in the *lrhA* mutant and loss of biofilm formation when LrhA was overexpressed. In *E. coli*, type 1 fimbriae as well as flagellar-mediated motility contribute to the initial stages of biofilm development on abiotic surfaces (Pratt & Kolter, 1998). Therefore, the effects of LrhA on biofilm formation are largely due to changed expression of flagella and type 1 fimbriae. Under the conditions tested, inhibition of fimbrial adhesion by methyl- α -D-mannopyranoside reduced biofilm formation even more drastically than lack of flagella, demonstrating the crucial role in biofilm formation of type 1 fimbriae and of their regulation by LrhA.

Fig. 4. Proposed model of regulation of type 1 fimbriae synthesis by LrhA. Activation of *fimE* expression by LrhA leads to repression of expression of the *fimA* operon.

Recently, the RNA-binding global regulatory protein CsrA (carbon storage regulator) has been reported to be a repressor of biofilm formation (Jackson *et al.*, 2002). In contrast to LrhA, CsrA apparently affects biofilm formation essentially through the regulation of glycogen metabolism, and not through alteration of surface-molecule expression. As expected, the CsrA-antagonizing regulatory RNA CsrB stimulated biofilm formation (Jackson *et al.*, 2002). Interestingly, HexA, a close relative of LrhA (>60% identity) from the plant pathogen *Erwinia carotovora*, negatively regulates the expression of *rsmB*, a homologue of *E. coli* *csrB* (Mukherjee *et al.*, 2000). One could therefore speculate that LrhA might repress biofilm formation both through the control of flagella and fimbriae synthesis as well as indirectly through carbon balance. Expression of *csrB* and *csrA*, however, was not affected by LrhA (unpublished results).

Virulence assessment of an *lrhA*-defective UPEC strain

Urinary tract infection is the major bacterial infectious disease in industrialized countries. Type 1, S and P fimbriae are typical virulence factors of UPEC. Fimbriae are involved in specific adherence to epithelia of the urogenital tract and promote both bacterial attachment to and/or invasion of host tissues within the urinary tract (Connell *et al.*, 1996; Keith *et al.*, 1986; Martinez *et al.*, 2000; Mulvey *et al.*, 1998). *In vivo* expression of type 1 fimbriae contributes to virulence in the urinary tract and *fim* expression is critical for infection of the lower urinary tract (cystitis) (Bahrani-Mougeot *et al.*, 2002; Gunther *et al.*, 2001).

Inactivation of *lrhA* results in an increased expression of genes encoding flagella and type 1 fimbriae both in the *E. coli* K-12 laboratory strain MG1655 and the UPEC strain 536. LrhA is closely related to HexA and PecT from plant-pathogenic *Erwinia* species, which function as repressors both of motility and of multiple virulence factors, such as lytic exoenzymes (Harris *et al.*, 1998; Surgey *et al.*, 1996). However, neither haemolytic activity nor other established virulence factors of the UPEC strain 536, such as S or P fimbriae, appear to be significantly affected by LrhA.

Thus, LrhA has a particular effect on type 1 fimbriae synthesis and hence on biofilm formation, but only a limited effect on the virulence of the UPEC strain as established in a murine model of ascending urinary tract infection. Other forms or models of virulence, in which type 1 fimbriae play a more important role, could depend more directly on LrhA.

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