Complex Interplay between Type 1 Fimbrial Expression and Flagellum-Mediated Motility of Uropathogenic Escherichia coli

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Type 1 fimbriae and flagella have been previously shown to contribute to the virulence of uropathogenic Escherichia coli (UPEC) within the urinary tract. In this study, the relationship between motility and type 1 fimbrial expression was tested for UPEC strain CFT073 by examining the phenotypic effect of fimbrial expression on motility and the effect that induction of motility has on type 1 fimbrial expression. While constitutive expression of type 1 fimbriae resulted in a significant decrease in motility and flagellum expression (P < 0.0001), a loss of type 1 fimbrial expression did not result in increased motility. Additionally, hypermotility and flagellar gene over- and underexpression were not observed to affect the expression of type 1 fimbriae. Hence, it appeared that the relationship between type 1 fimbrial expression and motility is unidirectional, where the overexpression of type 1 fimbriae dramatically affects motility and flagellum expression but not vice versa. Moreover, the constitutive expression of type 1 fimbriae in UPEC cystitis isolate F11 and the laboratory strain E. coli K-12 MG1655 also resulted in decreased motility, suggesting that this phenomenon is not specific to CFT073 or UPEC in general. Lastly, by analyzing the repression of motility caused by constitutive type 1 fimbrial expression, it was concluded that the synthesis and presence of type 1 fimbriae at the bacterial surface is only partially responsible for the repression of motility, as evidenced by the partial restoration of motility in the CFT073 fim L-ON ΔfimAFGCH mutant. Altogether, these data provide further insight into the complex interplay between type 1 fimbrial expression and flagellum-mediated motility.

Uropathogenic Escherichia coli (UPEC) causes the majority of urinary tract infections in otherwise healthy individuals. Studies suggest that up to 95% of all urinary tract infections develop in an ascending manner (3), beginning with periurethral colonization, followed by bladder infection (cystitis) and in some cases, if left untreated, ascension of the ureters to the kidney (pyelonephritis). Sequencing of the genome of pyelonephritis isolate E. coli CFT073 revealed 12 putative fimbrial gene clusters, including a type 1 fimbrial operon (60). UPEC utilizes type 1 fimbriae for adherence to N-linked oligomannose glycoproteins or uroplakin receptors found on transitional epithelia of the bladder (28, 46, 62). Our laboratory has established that type 1 fimbriae are highly expressed during murine urinary tract infection (54) and are important in infection, particularly in the first 24 h of experimental bladder colonization (22). Type 1 fimbriae have also been shown to satisfy molecular Koch’s postulates as a virulence factor (15).

The promoter upstream of the type 1 fimbrial operon resides within an invertible element (IE) of DNA that upon inversion changes the promoter orientation, which in turn affects the transcription of the fimAFGCH genes (1). The switching of promoter orientation ultimately leads to the expression (phase on) or loss of expression (phase off) of type 1 fimbriae and is mediated by the extensively studied FimB and FimE recombinases (8, 25, 41), in addition to the recently identified FimB- and FimE-like recombinases IpuA and IpbA (12). While FimB promotes both “on-to-off” and “off-to-on” phase variation, FimE has been shown to promote primarily “on-to-off” switching (19, 25).

While fimbria-mediated adherence is important during the initial stages of host colonization, bacterial motility and chemotaxis provide a means for UPEC to disseminate to new sites of the urinary tract to obtain nutrients when they become limited as well as to escape host immune responses. Recently, our laboratory and others have demonstrated that flagellum-mediated motility and chemotaxis also contribute to the fitness of UPEC during urinary tract colonization (32, 61). Genes for flagellum synthesis form an ordered and highly regulated cascade of three classes (26, 27, 38). The class I master regulon flhDC encodes a transcription factor necessary for class 2 gene transcription (38). Class 2 genes encode the basal body and hook of the flagellum in addition to FliA (σ26) and FlgM (anti-σ26 factor) (38). FliA is the sigma factor required for the transcription of the class 3 flagellar genes (29, 48). The class 3 genes encode hook-associated proteins and the filament of the flagellum (FlhC) as well as proteins necessary for motility and chemotaxis (such as CheY) (38). CheY is a response regulator (of a two-component regulatory system) that, upon phosphorylation, interacts with the flagellar motor switch complex and in turn influences the direction of flagellar rotation and speed of swimming (11, 42, 51, 53). Due to the fact that the flagellar filament of E. coli is highly immunogenic (59, 64) and that flagellum-mediated motility is very energy expensive (57), it is logical that flagellum synthesis be tightly regulated. This regulation is focused primarily at the level of the flhDC master regulon.

Reciprocal regulation between flagellum synthesis and fimbria production may allow bacteria to coordinate two counterproductive properties, motility and adherence. Indeed, it would not be advantageous for a bacterium tethered to an epithelial surface by
fimbriae to attempt swimming (or swarming) at a high rate of speed using its flagellar motor. Thus, it is intuitive that an adherent bacterium should not be highly motile and that a highly motile bacterium should be less adherent. Several genera of pathogenic and nonpathogenic bacteria have been shown to reciprocally regulate motility and adherence through different mechanisms. For example, the two-component BvgAS system of Bordetella pertussis acts as a switch between two states: the Bvg" phase activates the expression of adhesin and toxin genes, whereas the Bvg" phase promotes motility (17). In a different mode of regulation, mutations that alter motility in Vibrio cholerae directly feed back to the ToxR regulatory system to alter the expression of the toxin-co-regulated pilus (20).

To explore the potential relationship between motility and adherence of UPEC, we meticulously examined the connection between type 1 fimbrial expression, motility, and flagellum-mediated adherence during urinary tract pathogenesis. To present evidence that two UPEC strains constitutively producing type 1 fimbriae are significantly less motile and chemotactic to 1-asparteic acid than the wild type. Moreover, we demonstrate that this phenomenon is specific not only to UPEC but also to the laboratory E. coli strain CFT073. By deleting the fimAICDFGH genes in CFT073 fim L-ON (where the IE remains in the on position but the structural genes for type 1 fimbriae have been deleted), we observed a partial restoration of wild-type motility and chemotaxis to 1-asparteic acid, whereas the CFT073 fim L-ON variant must exist. Ultimately, data generated from these studies provide insight into the complex interplay between flagellum-mediated motility and a known adherence factor of E. coli. Furthermore, these findings aid in the understanding of the balance between motility and type 1 fimbria-mediated adherence during urinary tract pathogenesis.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** E. coli strain CFT073 was isolated from the blood and urine of a patient diagnosed with acute pyelonephritis. All E. coli strains and plasmids used in these studies are shown in Table 1. For initial propagation, all strains were grown on LB agar plates with appropriate contrast microscopy and using a capillary chemotaxis assay.

**TABLE 1. Bacterial strains and plasmids used in this study**

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<th>Strain or plasmid</th>
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**Note:** E. coli K-12 strain MG1655. Using transmission electron microscopy, Western blot analysis, and quantitative real-time reverse transcription-PCR (qPCR), we determined that CFT073 fim L-ON expresses significantly fewer flagella than wild-type CFT073. By deleting the fimAICDFGH genes in CFT073 fim L-ON (where the IE remains in the on position), we observed a partial restoration of wild-type motility and flagellum expression, thus suggesting that the expression of type 1 fimbriae at the bacterial surface is only partially responsible for the decrease in motility and flagellum expression observed for the CFT073 fim L-ON strain.

**Materials and Methods**

**Bacterial strains and culture conditions.** E. coli strain CFT073 was isolated from the blood and urine of a patient diagnosed with acute pyelonephritis. All E. coli strains and plasmids used in these studies are shown in Table 1. For initial propagation, all strains were grown on LB agar plates with appropriate contrast microscopy and using a capillary chemotaxis assay. We present evidence that two UPEC strains constitutively producing type 1 fimbriae are significantly less motile and chemotactic to 1-asparteic acid than the wild type. Moreover, we demonstrate that this phenomenon is specific not only to UPEC but also to the laboratory E. coli K-12 strain MG1655. Using transmission electron microscopy, Western blot analysis, and quantitative real-time reverse transcription-PCR (qPCR), we determined that CFT073 fim L-ON expresses significantly fewer flagella than wild-type CFT073. By deleting the fimAICDFGH genes in CFT073 fim L-ON (where the IE remains in the on position), we observed a partial restoration of wild-type motility and flagellum expression, thus suggesting that the expression of type 1 fimbriae at the bacterial surface is only partially responsible for the decrease in motility and flagellum expression observed for the CFT073 fim L-ON strain. Therefore, it is concluded that other unknown regulatory mechanisms, such as those that are upregulated when the fim switch is in the on orientation (irrespective of fim expression), that are also important for the reduction of motility and flagellum expression in the fim L-ON variant must exist. Ultimately, data generated from these studies provide insight into the complex interplay between flagellum-mediated motility and a known adherence factor of E. coli. Furthermore, these findings aid in the understanding of the balance between motility and type 1 fimbria-mediated adherence during urinary tract pathogenesis.
antibiotics (50 μg/ml nalidixic acid, 25 μg/ml kanamycin, or 100 μg/ml ampicillin) and incubated at 37°C for 18 h. Liquid cultures grown overnight were obtained by inoculating a single colony into LB broth containing appropriate antibiotics and incubation at 37°C for 18 h with aeration (200 rpm).

To culture wild-type and mutant strains of CFT073 in conditions that favor optimized wild-type motility, bacteria were cultivated from outer motility rings in 0.25% tryptone motility agar (see below) and grown in tryptone broth (10 g tryptone and 5 g NaCl per liter) at 30°C with aeration (200 rpm) for 16 h. Upon enriching for motile bacterial populations, the bacterial cultures were then stored for up to 1 week at 4°C. For the capillary chemotaxis assay, most Western blot analyses, transmission electron microscopy, and qPCR experiments, 400 μl of stored culture was resuspended into 12 ml of tryptone broth (10 g tryptone and 5 g NaCl per liter) in a 125-ml flask. Cultures were incubated at 30°C with aeration (200 rpm) until the optical density at 600 nm (OD600) reached ~0.3, corresponding to optimal wild-type motility. Motility was verified by viewing wet mounts of cultures by phase-contrast microscopy as described below. At this time, the cultures were immediately prepared for the capillary chemotaxis assay, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), transmission electron microscopy, or RNA extraction. For RNA preparation, 1 ml of RNAprotect bacterial reagent (QIAGEN) was added immediately to a 500-μl sample of bacterial culture to stabilize RNA according to the manufacturer’s specifications. After incubation at 25°C for 10 min, bacteria were harvested by centrifugation (10 min, 10,000 g) and the bacterial pellet was stored at -80°C for up to 4 weeks until RNA extraction.

Construction and characterization of CFT073 deletion mutants. Deletion mutants of flhDC, flhC, and fimAICDFGH were generated using the lambda red recombinase designed by Datsenko and Wanner (16) and as described previously for the flhC, flaA, flhM, and cheY deletion mutants (32). The flhDC and flhC deletion mutants were constructed in the wild-type CFT073 strain, while the fimAICDFGH deletion mutant was made in a type 1 fimbrial phase-locked deletion mutant (32). The primer sequences and descriptions are listed in Table 2.

Motility assays. Motility was initially determined by using soft agar plates. Specifically, 50 μl of a culture grown overnight was re-inoculated into 5 ml of sterile LB broth and incubated at 37°C with aeration (200 rpm) to an OD600 of 0.9 to 1.0. At that point, cultures were standardized to an OD600 of ~0.9 and stabbed into 0.25% tryptone agar plates using a sterile inoculating needle. The plates were incubated for 16 h at 30°C (or, for one experiment, at 37°C) before the diameter of swimming was assessed for each strain. Since the diameter of swimming in soft agar can be attributable to motility and chemotaxis, all motility agar results were confirmed by photomicrography. To do this, wet mounts of bacterial cultures, grown to an OD600 of ~0.3, corresponding to optimal wild-type motility, were viewed at ×400 magnification using a Zeiss Axioplan microscope. For both motility assays, wild-type CFT073 served as the positive control, and the nonmotile mutant CFT073 ΔflhC served as the negative control.

Capillary chemotaxis assay. A modified version of a standard capillary chemotaxis assay was performed as previously described (2, 31, 50). Bacteria were cultured for optimized wild-type motility as described above for the bacterial culture conditions. Cultures were harvested by centrifugation (2,000 × g, 10 min, 23°C), spent medium was aspirated, and bacteria were resuspended in chemotaxis buffer (10 mM potassium phosphate buffer [pH 7.0], 0.10 mM potassium EDTA [pH 8.0]) and incubated for 1 h at 30°C. Starved bacterial suspensions (500 μl) were added to the wells of the chambers, followed by the addition of maximal capillaries (1 μl) filled with either 10 mM L-aspargin (Sigma) or chemotaxis buffer as a control to measure random movement into the capillary. Chemotaxis chambers were incubated for 90 min at 30°C before capillaries were removed for serial dilution. Dilutions were spiral plated with an Autoplate 4000 apparatus (Spiral Biotech) and incubated at 37°C overnight. Colonies were enumerated using a Q-Count apparatus with automated colony-counting software (Spiral Biotech) to determine CFU/ml. L-Aspartate was diluted in chemotaxis buffer, adjusted to pH 8.0, to match that of chemotaxis buffer alone, 0.2-μm filter sterilized (Millipore), and stored at room temperature.

Cloning and overexpression of flhDC. To induce flagellar gene expression and motility, a 963-bp fragment containing the full open reading frame encoding FlhDC was cloned into pBAD/Myc-HisA (Invitrogen) under the control of the arabino-inducible promoter. Briefly, primers containing Ncol and PmeI restriction sites were designed to amplify and directionally clone flhDC directly downstream of the araBAD promoter, replacing the Myc-His region (Table 2). The resulting construct, pBAD-flhDC, and the empty pBAD vector were transformed into wild-type CFT073, CFT073 ΔflhDC, and CFT073 fimLON. Restriction digestion of isolated plasmid DNA was performed to confirm the presence and direction of the flhDC insert. The induction and repression of motility were determined for wild-type CFT073, CFT073 ΔflhDC, and CFT073 fimLON containing pBAD-flhDC or vector alone by subculturing into LB broth, cultivating the strains to an OD600 of ~0.8, and stabbing the standardized cultures into soft agar plates (1% tryptone, 0.5% NaCl, 0.25% agar) containing either 0.2%
arabinose (for induction) or 0.4% glucose (for repression). Plates were incubated for 16 h at 23°C before the degree of motility was assessed for each strain.

Induction of flhDC expression in the CFT073 fim L-ON strain containing pBAD-flhDC (and the empty pBAD vector as a control) was achieved by culturing bacteria in LB broth containing 100 μg/ml of ampicillin with shaking (200 rpm) at 37°C to an OD<sub>600</sub> of ~0.5 and then adding either 0.2% arabinose (for induction) or 0.4% glucose (for repression) to the cultures for induction or repression, respectively, of the araBAD promoter. At this point, the cultures were further incubated with shaking (200 rpm) at 37°C for an additional 2 h for complete induction or repression. Induction of flhDC expression in wild-type CFT073 was conducted differently, because the wild type does not constitutively express type 1 fimbriae, as does the fim L-ON strain, and does not optimally produce type 1 fimbriae under the growth conditions described above. Therefore, for optimal type 1 expression, wild-type CFT073 containing pBAD-flhDC (and the empty pBAD vector as a control) was cultured statically in LB broth containing 100 μg/ml of ampicillin for 48 h at 37°C and then subcultured (1:100) into fresh LB broth containing 100 μg/ml of ampicillin and cultured statically for an additional 48 h at 37°C. For the induction or repression of flhDC expression, 0.2% arabinose or 0.4% glucose, respectively, was added at the start of the initial culture and after every 24 h of culturing. After incubation, both wild-type CFT073 and the CFT073 fim L-ON mutant were prepared immediately for Western blot analysis or RNA extraction. For RNA preparation, 500 μl of culture was added to 1 ml of RNAProtect bacterial reagent (QIAGEN) and prepared as described above.

Transmission electron microscopy. Samples of wild-type CFT073 and the fim-looked mutants were obtained from the capillary chemotaxis assay (after they had been starved in phosphate-buffered saline for 1 h at 30°C). Carbon-coated copper grids were coated with 1% sodium phosphotungstic acid (pH 5.8) for 30 s. The grids were examined with a Philips CM-100 transmission electron microscope at an operating voltage of 60 kV. Bacteria were examined between the magnifications of ×7,900 and ×25,000. Digital images of bacteria were captured with an automated computer-controlled and a Kodak 1.6 Megaplus high-resolution digital camera. All materials, kit base solutions, and solutions, unless otherwise noted, were obtained from Electron Microscopy Sciences (Fort Washington, PA).

Detection of flagellum and fimbria expression. For the wild type, the fim-looked mutants, and the flagellar mutants, bacteria were cultured for optimized wild-type motility as described above in bacterial culture conditions. Whole-cell lysates were prepared for electrophoresis by adding either an equal volume of 2× SDS sample buffer (flagellum samples) or 2× SDS sample buffer acidified with 1 N HCl (fimbria samples), followed by boiling for 10 min. After boiling, the fimbrial samples were neutralized with 1 N NaOH. Samples were electrophoresed using discontinuous one-dimensional SDS-PAGE as described previously by Laemmli (30). After electrophoresis, gels were transferred onto a polyvinylidene difluoride membrane (Immobilon-P, Millipore Corp.). Western blots for the detection of flagella were incubated with a 1:40,000 dilution of rabbit polyclonal antisera to FlhC (Sigma) to enhance the attachment of bacteria to the grid. A droplet (8 μl) of culture was placed onto the grid for 5 min. Bacteria were then fixed with 2.5% glutaraldehyde and stained with 1% sodium phosphotungstic acid (pH 5.8) for 30 s. The grids were examined with a Philips CM-100 transmission electron microscope at an operating voltage of 60 kV. Bacteria were examined between the magnifications of ×7,900 and ×25,000. Digital images of bacteria were captured with an automated computer-controlled and a Kodak 1.6 Megaplus high-resolution digital camera. All materials, kit base solutions, and solutions, unless otherwise noted, were obtained from Electron Microscopy Sciences (Fort Washington, PA).

RESULTS

Constitutive expression of type 1 fimbriae results in decreased motility and chemotaxis to l-aspartate in UPEC strain CFT073. Two surface organelles, flagella and fimbriae, are important in microbial pathogenesis but mediate opposite and likely antagonistic actions. We hypothesized that UPEC reciprocally regulates motility and adherence. Previously, our laboratory constructed type 1 fimbrial phase-locked derivatives of CFT073 that bear a mutation in the left inverted repeat of the type 1 fimbrial IE that blocks DNA inversion by FimB and FimE, thus resulting in either a locked-on (fim L-ON) or locked-off (fim L-OFF) promoter orientation (22). To determine the effect that type 1 fimbrial expression has on motility, wild-type strain CFT073 and the fim L-ON phase-locked derivative of CFT073 that constitutively expresses type 1 fimbriae were evaluated for their motility in 0.25% tryptone soft agar. The average diameter of swimming of CFT073 fim L-ON was dramatically less (about fourfold; P < 0.0001) than that of wild-type CFT073 (Fig. 1A, C, and G). A flagellum-negative mutant, CFT073 ΔflhC, used as a negative control, was nonmotile in 0.25% soft agar (Fig. 1B). Since the diameter of swimming in soft agar can be attributable to both motility and chemotaxis, the repression of motility in CFT073 fim L-ON was confirmed by phase-contrast microscopy. Indeed, we observed that CFT073 fim L-ON was much less motile than wild-type CFT073 and CFT073 fim L-OFF (a phase-locked derivative of CFT073 unable to produce type 1 fimbriae). Although the expression of type 1 fimbriae has been shown to be poorly expressed, if at all, by E. coli at temperatures lower than 37°C owing to the differential temperature modulation by H-NS of the fimB and fimE genes (49), we demonstrate by transmission electron microscopy and Western blot analysis that CFT073 fim L-ON (phase-locked mutant that constitutively expresses type 1 fimbriae and cannot undergo FimB and FimE recombination) is heavily fimbriated and abundantly expresses FimH at 30°C throughout the motility assays (Fig. 2B and D, respectively). Therefore, it is possible that the presence and abundance of type 1 fimbriae at the bacterial surface may be contributing to the reduction in motility observed in the fim L-ON variant. To determine whether these results would be consistent at higher temperatures that favor type 1 fimbrial
expression, we also compared the motilities of wild-type CFT073 and CFT073 ΔfliC in 0.25% tryptone soft agar after incubation for 16 h at 37°C. Once more, we observed that the average motility diameter of the wild type (44 ± 4 mm) was significantly greater than the average motility diameter observed for CFT073 Δfim L-ON (8 ± 2 mm) (*P < 0.0001) (data not shown).

In view of the fact that the CFT073 Δfim L-ON mutant was shown to have reduced motility, it was not surprising that it was also observed to be significantly less chemotactic to L-aspartate than wild-type CFT073 by using a capillary chemotaxis assay (Fig. 1H) (*P < 0.0001). To account for the random movement of bacteria into the capillaries during the chemotaxis assay, wild-type CFT073 chemotaxis to buffer alone (with no aspartate) was used as a negative control (Fig. 1H). As expected, there were significantly more wild-type CFT073 bacteria present in the capillaries filled with aspartate than with buffer alone (Fig. 1H) (*P < 0.0001).

Constitutive expression of type 1 fimbriae results in decreased flagellin expression and transcription of fliC in UPEC strain CFT073. To determine whether the reduced motility of CFT073 Δfim L-ON was due to a decrease in flagellum expression, wild-type CFT073 and the CFT073 Δfim L-ON mutant were cultured in tryptone broth with aeration to a density corresponding to optimal motility (for the wild type, an OD600 of ~0.3). The production of flagella was initially assessed by Western blot analysis using H1 flagellin antiserum. To verify that CFT073 Δfim L-ON was still producing type 1 fimbriae under these conditions, which were not optimal for type 1 fimbria expression, the same lysates were subjected to Western blot analysis using FimH antiserum. As expected, wild-type CFT073 did not produce detectable levels of type 1 fimbriae, whereas strain CFT073 Δfim L-ON was shown to express abundant FimH (Fig. 2D). The increased expression of type 1 fimbriae correlated with a reduction in flagellin expression in CFT073 Δfim L-ON compared to wild-type CFT073 (Fig. 2D).

Levels of fimbriation and flagellation were also assessed by transmission electron microscopy. While wild-type CFT073 was frequently observed to be flagellated, CFT073 Δfim L-ON was rarely observed to produce flagella (Fig. 2A and B, respectively, black arrowheads). Conversely, wild-type CFT073 was rarely observed to express fimbriae, whereas CFT073 Δfim L-ON was observed to express an abundant amount of fimbriae (presumably type 1 fimbriae) (Fig. 2A and B, respectively, white arrowheads). As a negative control, the CFT073 ΔfliC mutant was never observed to produce flagella (data not shown).

To determine whether the decrease in flagellin expression is regulated at the level of transcription, the amounts of fliC transcripts between wild-type CFT073 and the CFT073 Δfim L-ON mutant were compared using qPCR. Consistent with the Western results, the level of fliC transcripts in the CFT073 Δfim L-ON strain was decreased 46-fold (n = 3) relative to wild-type CFT073 (Fig. 2E). Additionally, the levels of fimA transcripts were observed to be 85-fold (n = 3) greater in the CFT073 Δfim L-ON mutant than in wild-type CFT073, thus confirming that this mutant constitutively expresses type 1 fimbriae (Fig. 2E).

Overall, these data were consistent with an inverse relationship between type 1 fimbria and flagellum expression.

Loss of type 1 and P fimbrial expression does not have an inverse effect on motility in UPEC strain CFT073. To further examine the effect of fimbrial expression on motility, we analyzed the ability of various fimbrial mutants of CFT073 to swim in 0.25% soft agar. Based on the reciprocal nature of flagellum
and fimbria expression of the CFT073 fim L-ON strain, we hypothesized that the loss of fimbrial production would result in increased flagellation and motility. Surprisingly, we noticed a slight reduction (not increase) of CFT073 fim L-OFF motility and chemotaxis compared to wild-type CFT073 (Fig. 1A, D, G, and H). Likewise, a double pap mutant (UPEC76) and triple fim pap mutant of CFT073 were also shown to be slightly less motile than the wild type in soft agar (Fig. 1A, E, F, and G).

The decrease in motility of the CFT073 fim L-OFF variant also correlated with a decrease in FliC expression as assessed by Western blot analysis using H1 flagellin antiserum (Fig. 2D). Moreover, the level of fliC transcription in the CFT073 fim L-OFF mutant was observed to be 23-fold less than that in wild-type CFT073 (Fig. 2E). Although the CFT073 fim L-Off mutant displayed only a slight decrease in motility compared to the wild type, it is interesting that a dramatic decrease in flagellin expression was observed. It is possible that the expression of FliC in a motile liquid culture is not fully representative of FliC expression in motility agar. However, a general trend that decreases in motility always correlated with decreases in FliC expression and vice versa was always observed.

To further investigate the slight reduction of motility exhibited by the CFT073 fim L-Off mutant, we used transmission electron microscopy to visualize the surface expression of flagella and fimbriae. As observed with the Western analyses and qPCR, CFT073 fim L-Off appeared to be less flagellated than wild-type CFT073 (Fig. 2A and C, black arrowheads). Interestingly, however, the CFT073 fim L-Off mutant was observed to produce more fimbriae than wild-type CFT073 (Fig. 2A and C, white arrowheads). We demonstrate in Fig. 2D that the fimbriae produced by CFT073 fim L-Off are not type 1 fimbriae. This is not too surprising, since previous studies from our laboratory have demonstrated that a loss of one fimbrial type leads to the expression of another in UPEC (33, 55). Therefore, it is possible that the increased fimbria production of the fim L-Off mutant may be responsible for its decreased motility. Also, the slight decreases in motility observed for CFT073 fim L-Off, UPEC76, and CFT073 Δfim pap may not be as biologically significant as the dramatic decrease in motility observed for the CFT073 fim L-Off mutant (a strain that is forced to constitutively produce type 1 fimbriae). Thus, flagellum expression and motility appear to be mostly affected...
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Induction of flhDC expression does not affect expression of type 1 fimbriae in UPEC strain CFT073. To examine the effect that a motility phenotype has upon type 1 fimbria expression, we introduced the flagellar class I master regulon flhDC under the inducible/repressible arabinose promoter contained on a plasmid (pBAD-flhDC). When introduced into an flhDC mutant of CFT073, the induction of pBAD-flhDC with 0.2% arabinose restored motility in soft agar and FliC expression (data not shown). Interestingly, the induction of pBAD-flhDC with 0.2% arabinose also resulted in increased motility of the CFT073 flm L-ON mutant in soft agar, which was repressed upon the addition of 0.4% glucose (Fig. 3C and D). Therefore, these data demonstrated that the induced expression of flhDC on a plasmid could reliably create a hypermotile state by activating the hierarchical flagellar machinery within the bacterial cell.

Since we observed a decrease in motility of the CFT073 flm L-ON mutant, we initially monitored the amounts of FlIC and FimH expressed on the surface of CFT073 flm L-ON containing either the empty pBAD vector or pBAD-flhDC (in the presence of 0.2% arabinose or 0.4% glucose) by Western blot analysis. As the expression of FlIC increased in the CFT073 flm L-ON mutant containing pBAD-flhDC (induced with 0.2% arabinose), the levels of FimH expression were comparable to that of CFT073 flm L-ON containing either pBAD-flhDC (repressed with 0.4% glucose) or the empty pBAD vector (Fig. 3E). Since it is possible that the CFT073 flm L-ON mutant may have stably produced type 1 fimbriae prior to the induction of flhDC expression, we also determined the level of FimH expression of wild-type CFT073 during the induction of pBAD-flhDC. For these experiments, we induced flhDC expression during serial passage of static culture in LB medium for optimal type 1 fimbrial expression. The induction of pBAD-flhDC with 0.2% arabinose in wild-type CFT073 consistently resulted in increased FimH expression as assessed by Western blot analysis using H1 flagellin antiserum (Fig. 3E). On the other hand, we observed that the induction of flhDC in wild-type CFT073 did not result in a decrease of FimH expression compared to wild-type CFT073 containing the empty pBAD vector (Fig. 3E). While the constitutive expression of type 1 fimbriae results in decreased motility and FlIC expression, the inverse does not appear to be true; thus, induced expression of FlIC and motility does not result in decreased type 1 fimbria expression.

Class I, II, and III flagellar mutants do not upregulate type 1 fimbrial expression in UPEC strain CFT073. Finally, to fully characterize the relationship between type 1 fimbria expression and motility, we investigated the possible effect of flagellar mutation on type 1 fimbrial expression. In particular, we hypothesized that flagellar mutants of CFT073 may upregulate type 1 fimbrial expression. For this study, isogenic flagellar mutants of CFT073 carrying disruptions in various flagellar genes within the three different hierarchical classes were cultured in tryptone broth with aeration to the early exponential phase for optimal motility (although mutants remained nonmotile) and then examined by a type 1 fimbrial IE assay and Western blot analysis for the expression of type 1 fimbriae. The IE PCR assay was conducted as described previously (36). For the IE assay and Western analysis, the CFT073 flm L-ON and flm L-OFF mutants were used as positive and negative controls, respectively, for type 1 fimbrial expression. Wild-type CFT073 and each flagellar mutant appeared to have their type 1 fimbrial expression. Therefore, it seems that overall, motility and flagellar gene over- and underexpression do not affect the expression of type 1 fimbriae.

Constitutive expression of type 1 fimbriae results in decreased motility in other E. coli strains. The majority of E. coli strains express type 1 fimbriae and are capable of flagellum-mediated motility (24, 38). Therefore, to examine whether the repression of motility during constitutive expression of type 1 fimbriae is a phenomenon specific to UPEC strain CFT073, we examined the motility of phase-locked type 1 fimbrial variants of other E. coli strains: the cystitis isolate F11 (56) and the laboratory K-12 strain MG1655 (40). As observed with CFT073 flm L-ON, the motility of F11 flm L-ON was significantly lower than that of wild-type F11 (Fig. 5A, B, and G) (P < 0.0001), indicating that at least one other UPEC isolate exhibits the same repression-of-motility phenotype during constitutive expression of type 1 fimbriae. Also, this phenotype does not appear to be specific to UPEC, in that the motility of the flm L-ON variant of the laboratory K-12 strain MG1655 was also significantly decreased compared to that of the K-12
fim L-OFF strain (Fig. 5D, E, and G). Interestingly, the F11 fim L-OFF mutant was observed to be slightly less motile than wild-type F11 (Fig. 5A, C, and G), which was similar to that seen with the CFT073 fim L-OFF mutant (Fig. 1A, D, and G). On the other hand, the K-12 fim L-OFF mutant appeared to be as motile as wild-type K-12. This result suggests that other factors that inhibit motility in CFT073 and F11 may not be present or expressed in K-12. However, as mentioned above, the differences in motility of the fim L-OFF mutants are not as biologically significant as those seen with the fim L-ON mutants.

Deletion of the type 1 fimbrial operon of CFT073 fim L-ON and its effect on motility. Motilities of wild-type (WT) CFT073 (A), CFT073 fim L-ON (B), and CFT073 fim L-ON ΔfimAICDFGH (fimA-H) (C) in soft agar are shown. Standardized cultures of each strain were stabbed into 0.25% soft agar plates and incubated for 16 h at 30°C. (D) Black bars represent the average diameters of swimming in mm) of triplicate motility plates. Error bars represent the SEM. Significant differences in motility (comparing the wild type to fim L-ON and fim L-ON ΔfimAICDFGH) were determined using an unpaired Student's t test with Welch correction (Instat; GraphPad). * P < 0.0001.
clude that the constitutive expression of type 1 fimbriae partially represses motility and fliC expression in CFT073 fim L-ON, but the appearance of the fimbriae on the surface of the bacteria is not solely responsible for this phenotype.

**DISCUSSION**

The objective of these studies was to examine a possible connection between the adherence and motility of UPEC pyelonephritis strain CFT073 in order to gain further insight into the interplay between these two important pathogenic traits. Initially, we hypothesized that motility and adherence were reciprocally regulated, since it would clearly be disadvantageous for a bacterium to stick and swim at the same time. Since flagella are organelles that are generally used for motility, and fimbriae are utilized for adherence, we presumed that these two surface structures would be reciprocally expressed by an individual bacterium. Recently, it has been demonstrated that unlinked FimB- and FimE-like recombinase mutants in the fim switch-on orientation are nonmotile in soft agar, while recombinase mutants in the fim switch-off orientation are motile (12).

In parallel, our laboratory showed that the constitutive expression of type 1 fimbriae results in decreased motility, chemotaxis to L-aspartate, and decreased flagellum expression; however, flagellar mutants deficient in motility or chemotaxis did not upregulate the expression of type 1 fimbriae (33a). Here, we examined the relationship between type 1 fimbria, motility, and flagellum expression in detail.

As type 1 fimbriae are thought to promote the autoaggregation of *E. coli* (52), we thought it possible that type 1 fimbriae may contribute to the autoaggregation of CFT073 fim L-ON, thus physically limiting its ability to swim in soft agar. However, upon further investigation, we noted that autoaggregation could not be solely responsible for the reduced motility of CFT073 fim L-ON in that CFT073 fim L-ON produced significantly fewer fliC transcripts than wild-type CFT073 (Fig. 2A). Therefore, it is most likely that the down-regulation of fliC transcription is the cause of the decrease in motility. Nevertheless, it is possible that autoaggregation of strain CFT073 fim L-ON may result in some feedback regulatory mechanism that results in decreased fliC transcription. If this were the case, further studies would be needed to characterize the potential role of autoaggregation in the reduction of motility.

While the constitutive expression of type 1 fimbriae resulted in a significant decrease in motility and flagellin expression, the loss of type 1 and P fimbrial expression did not result in increased motility. In fact, both of the UPEC fim L-OFF mutants, as well as UPEC fimL-ON and the CFT073 fim paf mutant, displayed slight decreases in motility compared to wild-type CFT073 and F11. Upon further investigation, we observed that the CFT073 fim L-OFF mutant produces more fimbriae than wild-type CFT073 by transmission electron microscopy. This is consistent with previous studies from our laboratory and others that have shown that the loss of one fimbrial type generally leads to the expression of another fimbrial type (33). In particular, Snyder et al. previously demonstrated that the loss of both type 1 and P fimbriae in UPEC pyelonephritis strain CFT073 leads to the expression of F1C fimbriae (55). A similar study conducted previously by Wright et al. demonstrated that the loss of type 1 fimbriae in UPEC cystitis strain UTI89 led to the increased expression of S fimbriae (61a). Therefore, it is possible that the loss of type 1 or P fimbrial expression leads to the production of another fimbrial type, which in the end may be the cause of the slight decrease (and not increase) in motility in the fim L-OFF (and other fimbrial deletion mutant) strains. Since these strains are still able to shut off and regulate fimbrial expression, we speculate that this is the reason why their motility is not as significantly or drastically reduced as that of the fim L-ON strains, which are forced to constitutively express type 1 fimbriae. Ultimately, we conclude that flagellum expression and motility appear to be mostly affected when there is an abundance of fimbria expression and not a loss of fimbria expression.

As there are several studies that demonstrate a reciprocal balance between motility and adherence (14, 17, 20, 35), there are also a few studies that provide evidence that is inconsistent with our original hypothesis of reciprocal regulation. For instance, while flagella are more thoroughly documented for their involvement in motility and fimbriae for their adhesive-ness, there is also evidence for type IV pilus-mediated twitching motility and flagellum-mediated adherence (21, 39). Additionally, flagella and type 1 fimbriae have been shown to be integral components of adherent-invasive *E. coli* strain LF82 in the adherence to and invasion of intestine-407 cells (4, 10). In particular, Barnich et al. previously revealed that flagella and type 1 fimbriae of LF82 are regulated in a coordinate manner, as evidenced by the reduction of type 1 fimbria expression in a fliC mutant, and went on to speculate that flagella, type 1 fimbriae, and other unknown factors coordinately contribute to the invasive ability of adherent-invasive *E. coli* strain LF82 (4). It has also been shown that both fimbra- and flagellum-mediated motility are important for the association and invasion of cultured epithelial cells by *Salmonella enterica* serovar Enteritidis (18). Therefore, it is possible that both adherence and motility act in unison during different stages of colonization, including invasion of and attachment to host cells.

Finally, in this report, we demonstrate a unidirectional relationship between flagellum-mediated motility and type 1 fimbrial expression (and presumably adherence) that appears to be common among motile strains of both UPEC and other *E. coli* strains. In particular, we observed that type 1 fimbrial phase-locked derivatives of UPEC strains CFT073 and F11 as well as *E. coli* K-12 strain MG1655 that constitutively express type 1 fimbriae (fim L-ON) are much less motile and express fewer flagella than their respective wild-type or fim L-OFF strains. Moreover, we demonstrated that the decrease in motility of the fim L-ON strains occurs at the level of fliC transcription. By examining a fim null mutant of CFT073 fim L-ON, we observed a significant increase in motility compared to the fim L-ON parent; however, the level of motility was still significantly lower than that of wild-type CFT073. This observation led us to conclude that the presence of type 1 fimbriae on the surface of the bacterium is not solely responsible for the decreased motility of CFT073 fim L-ON. Indeed, it is possible that factors that influence the orientation or induction of the fim promoter, rather than the physical presence of fimbriae at the bacterial surface, also contribute to the repression of motility. This is intriguing because there are a number of transcriptional regulators, including LrhA (9, 34), Lrp (7, 13, 23, 47), H-NS (5, 49), and IHF (8, 63), that have been shown to...
modulate the expression of both flagella and fimbrirae. Currently, our laboratory is in the process of determining the mechanism(s) of regulation that results in the decreased motility observed in the fim L-ON strain.

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