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Characterization of 17 chaperone-usher fimbriae encoded by *Proteus mirabilis* reveals strong conservation

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Proteus mirabilis is a Gram-negative enteric bacterium that causes complicated urinary tract infections, particularly in patients with indwelling catheters. Sequencing of clinical isolate *P. mirabilis* HI4320 revealed the presence of 17 predicted chaperone-usher fimbrial operons. We classified these fimbriae into three groups by their genetic relationship to other chaperone-usher fimbriae. Sixteen of these fimbriae are encoded by all seven currently sequenced *P. mirabilis* genomes. The predicted protein sequence of the major structural subunit for 14 of these fimbriae was highly conserved (⩾95 % identity), whereas three other structural subunits (Fim3A, UcaA and Fim6A) were variable. Further examination of 58 clinical isolates showed that 14 of the 17 predicted major structural subunit genes of the fimbriae were present in most strains (>85 %). Transcription of the predicted major structural subunit genes for all 17 fimbriae was measured under different culture conditions designed to mimic conditions in the urinary tract. The majority of the fimbrial genes were induced during stationary phase, static culture or colony growth when compared to exponential-phase aerated culture. Major structural subunit proteins for six of these fimbriae were detected using MS of proteins sheared from the surface of broth-cultured *P. mirabilis*, demonstrating that this organism may produce multiple fimbriae within a single culture. The high degree of conservation of *P. mirabilis* fimbriae stands in contrast to uropathogenic *Escherichia coli* and *Salmonella enterica*, which exhibit greater variability in their fimbrial repertoires. These findings suggest there may be evolutionary pressure for *P. mirabilis* to maintain a large fimbrial arsenal.

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INTRODUCTION

Urinary tract infections (UTIs) are the most common bacterial infections in humans (Foxman, 2003). In 2006, UTIs were the cause of over eleven million medical consultations, as well as nearly half a million hospitalizations in the US alone (Litwin & Saigal, 2007; DeFrances *et al.*, 2008). Worldwide, UTIs are also the most prevalent type of healthcare-associated infection (Tambyah, 2004). Most of these infections are associated with the use of urinary catheters (catheter-associated UTI) (Tambyah & Maki, 2000). In the US, an estimated 13 088 fatal healthcare-associated infections were due to UTIs in 2002 (Klevens *et al.*, 2007).

Proteus mirabilis is a bacterial pathogen that is associated with complicated UTI, particularly during long-term urinary catheterization [reviewed by Nielubowicz & Mobley (2010) and Armbruster & Mobley (2012)]. *P. mirabilis* infection can result in cystitis and often progresses to pyelonephritis. This member of the *Enterobacteriaceae* produces many virulence factors (Nielubowicz & Mobley, 2010), including urease, which hydrolyses urea in the urine, thereby increasing local pH. The alkaline environment causes the formation of struvite and apatite crystals, which contribute to stone formation (Griffith *et al.*, 1976; Mobley *et al.*, 1995).

A critical component of *P. mirabilis* pathogenesis centres on fimbriae (also known as pili) and other adhesins, which mediate adherence to cells of the urinary tract, as well as catheters (Wray *et al.*, 1986; Roberts *et al.*, 1990; Jansen *et al.*, 2004; Alamuri *et al.*, 2010). Notably, in a survey of Gram-negative bacteria, this species was found to have the greatest ability to adhere to catheters (Roberts *et al.*, 1990). Before sequencing and annotation of the *P. mirabilis* clinical isolate HI4320 genome (Pearson *et al.*, 2008), five *P. mirabilis*-encoded fimbriae had been identified: mannose-resistant *Proteus*-like (MR/P), *P. mirabilis* fimbria (PMF), ambient temperature fimbria (ATF), urothelial cell adhesin (UCA) (or non-agglutinating fimbria, NAF) and *P. mirabilis*

Abbreviations: NYULMC, New York University Langone Medical Center; qRT-PCR, quantitative reverse transcriptase PCR; UTI, urinary tract infection.

Five supplementary tables are available with the online version of this paper.

P-like pili (PMP) (Old & Adegbola, 1982; Wray *et al.*, 1986; Bahrani *et al.*, 1994; Massad *et al.*, 1994a, b; Bijlsma *et al.*, 1995). A sixth fimbria, MR/K, has been proposed based on the ability of *P. mirabilis* to agglutinate specific erythrocytes, but the genes responsible for this phenotype have not yet been identified (Old & Adegbola, 1982). Electron microscopy and immunoblot analyses have demonstrated that *P. mirabilis* can produce two fimbriae concurrently (Old & Adegbola, 1982; Adegbola *et al.*, 1983; Bahrani & Mobley, 1993; Tolson *et al.*, 1995; Zunino *et al.*, 2007). However, those experiments were not designed to detect and identify a wider variety of fimbriae. Sequencing of the HI4320 genome led to the discovery of 17 potential chaperone-usher fimbrial operons, an additional 12 operons compared with what was known previously. Little is known about these predicted fimbriae, including whether or not they are functional adhesins.

Studies of fimbriae in other Gram-negative bacteria have shown that most fimbriae are generally not conserved within a given species. For example, in *Escherichia coli*, only 3 of 15 different fimbriae were detected in greater than 40 % of commensal isolates tested. Although more types of fimbriae are typically found in pathogenic *E. coli* isolates, only 5 of 15 fimbriae were present in greater than 60 % of urinary isolates (Spurbeck *et al.*, 2011). Similarly, for *Salmonella enterica*, a species that infects a broad host range, there is considerable variation in the presence of fimbrial genes within different subspecies (Townsend *et al.*, 2001). However, there is greater conservation of fimbrial genes within the human- and higher primate-specific *S. enterica* serovar Typhi. Other studies have suggested that four fimbriae of *P. mirabilis* (ATF, PMF, MR/P and UCA) are conserved in limited numbers of clinical or environmental isolates (Bahrani & Mobley, 1994; Gaastra *et al.*, 1996; Massad *et al.*, 1996; Zunino *et al.*, 2000, 2003).

Since fimbriae often contribute to virulence, we sought to determine whether fimbriae encoded by *P. mirabilis* were broadly conserved or whether particular types were associated with specific isolation sites. Furthermore, we explored whether all 17 fimbrial operons are transcribed, and if so, how they are regulated. We have therefore analysed all 17 chaperone-usher fimbriae of *P. mirabilis* using DNA-, RNA- and protein-based approaches.

METHODS

Bacterial strains and media. A collection of clinical *P. mirabilis* strains was obtained during the early 1980s from two nursing homes in Baltimore, MD, USA, from patients with chronic indwelling catheters. Among these isolates was strain HI4320, which was isolated from the urine of a long-term ($\geqslant$30 days) catheterized elderly woman with bacteriuria (Warren *et al.*, 1982). NYU strains were collected from Tisch Hospital at New York University Langone Medical Center (NYULMC), NY, USA, from July 2012 to October 2012. The collection consists of tribe *Proteeae* (genera *Proteus*, *Providencia* and *Morganella*) strains identified by the Clinical Microbiology Laboratory during this time. All strains were grown in Luria Broth (LB; 10 g tryptone l^{-1}, 5 g yeast extract l^{-1}, 0.5 g NaCl l^{-1}) or on LB solidified with 15 g agar l^{-1}. Bacteria were routinely cultured at 37 °C.

To measure expression of fimbrial genes, *P. mirabilis* was cultured under the following conditions: exponential phase at 37 °C with aeration to an OD_{600} of 0.8. For experiments testing the effect of pH on fimbrial gene expression, MOPS was added to LB to a final concentration of 100 mM and the pH adjusted to 6.0 or 8.0. This buffering capacity was sufficient to maintain cultures near the target pH: at the time of collection, the pH 6.0 cultures were pH 6.10–6.13, while the pH 8.0 cultures were pH 7.86–7.89. Stationary-phase aerated cultures were harvested after 8 h (OD_{600}=3.86 to 4.50) and stationary-phase static cultures were harvested after 24 h. Bacterial colonies were collected from the surface of LB agar plates using a sterile loop after incubation for 16 h. Once the appropriate time point or OD_{600} was reached, 500 µl (one volume) of cells was added to 1 ml (two volumes) of RNAprotect (Qiagen); colonies were added directly to RNAprotect. After 10 min, the bacteria were centrifuged for 10 min at 6000 ***g*** and the bacterial pellet was stored at −20 °C prior to RNA extraction.

Comparison of sequenced genomes. BLAST (Altschul *et al.*, 1990) was used to detect predicted fimbrial genes in six recently sequenced *P. mirabilis* genomes. These genomes were sequenced by several independent groups and are available in GenBank at the following accession numbers: ATCC 29906 (ACLE01000000), WGLW4 (AMGU01000000), WGLW6 (AMGT0100000), BB2000 (NC_010555.1), C05028 (ANBT01000000) and PR03 (AORN01000000). Of these, BB2000 and PR03 are published (Khalid *et al.*, 2013; Sullivan *et al.*, 2013). The Kyoto Encyclopedia of Genes and Genomes (KEGG) (Tanabe & Kanehisa, 2012) and xBASE (Chaudhuri *et al.*, 2008) were used to analyse operon organization for HI4320, ATCC 29906 and BB2000, and genomic location of fimbrial operons for HI4320 and BB2000.

Detection of fimbrial genes. Genomic DNA was extracted from the strains from the 1980s isolates using the Generation capture column kit (Qiagen) and PCR was performed using primers targeting the predicted major structural subunits for all 17 fimbriae. Colony PCR was performed to detect genes in NYU clinical isolates. In both cases, negative or inconclusive results were tested at least twice, and if results remained inconclusive, genomic DNA was extracted and PCR was performed with genomic DNA as the template. All strains with negative results were rescreened using a second set of primers targeting the same genes. Primers used are listed in Table S1 (available in the online Supplementary Material).

Relatedness of isolates. Primers that uniquely target the *mrpA* and *flaA* (PMI1620) genes were used to PCR amplify these genes from five NYU clinical isolates. A total of 82 and 83 % of each gene was amplified, respectively. Each PCR product was sequenced (Genewiz). The resulting sequences were aligned by the CLUSTAL W method and plotted on a phylogenetic tree using MEGALIGN software (DNASTAR v. 10.1.1).

RNA extraction, reverse transcriptase PCR and quantitative reverse transcriptase PCR (qRT-PCR). The RNeasy minikit (Qiagen) was used to extract RNA with the following modification: bacteria were incubated with 3 mg lysozyme ml^{-1} instead of 1 mg lysozyme ml^{-1}, and placed on a vortex at a low setting at room temperature for 15 min. RNA was then treated with DNase (Ambion) according to the manufacturer's instructions.

The Superscript First-Strand synthesis system (Invitrogen) was used to generate cDNA from RNA according to the manufacturer's instructions. PCR using primers targeting *rpoA* (RNA polymerase A) with cDNA templates with or without reverse transcriptase treatment was used to confirm lack of genomic contamination. cDNA was then

purified and concentrated using a plasmid miniprep kit (Zymo) with the following modifications: PB buffer (Qiagen) was used to bind cDNA to the columns, and steps 9–11 from the Zymo plasmid miniprep kit were performed according to the manufacturer's instructions.

qRT-PCR was performed to test the expression of the 17 fimbriae in duplicate 25 µl reactions using 30 ng cDNA template, 150 nM each primer (Table S1) and 12.5 µl Maxima SYBR Green/fluorescein qPCR master mix (Thermo Scientific). Amplification of specific genes was performed over 40 cycles with a CFX Connect thermal cycler (Bio-Rad). No-template controls and melting curve analysis were used to confirm the absence of genomic DNA and determine the presence of primer dimers. Data were analysed using the threshold cycle ($2^{-\Delta\Delta C_T}$) method (Livak & Schmittgen, 2001) and normalized to *rpoA*. At least three independent experiments were analysed for each condition; for stationary-aerated culture and stationary-static culture, four or five experiments were conducted, respectively. Statistical analysis was performed using a two-tailed *t*-test with GraphPad Prism software, v. 5, on $\log_2$ transformed data.

Mass spectrometry. *P. mirabilis* HI4320 cultured overnight at 37 °C with aeration was diluted 1 : 100 into LB and grown at 37 °C with aeration for 8 h. Extracellular proteins were sheared from the cell surface by vigorously shaking the culture for 5 min. Cells were pelleted by centrifugation; supernatants were then sterilized with a 0.22 µM pore filter (Millipore) and concentrated ~100-fold using a centrifugal filtration unit with a 10 kDa filter (Millipore). To dissociate assembled fimbriae, samples were mixed 1 : 1 with HCl-acidified water (pH 1.8), boiled for 15 min and neutralized using NaOH.

Sheared proteins were sent to the NYULMC Proteomic Core for MS. Proteins were reduced with 10 mM DTT and alkylated with 25 mM iodoacetamide and loaded into a 4–12 % Bis-Tris gel (Life Technologies). The gel was run for 1.5 min at 200 V, fixed with 16 % methanol in 1 % acetic acid and stained with GelCode blue stain reagent (Thermo Scientific). The portion of the gel that contained all proteins was excised, destained and trypsin digested (200 ng) overnight. Then, peptides were extracted using R2 20 µm Poros beads (Life Technologies) as described elsewhere (Cotto-Rios *et al.* 2012). Approximately 7 µg of the peptide mixture was loaded onto a Acclaim PepMap 100 precolumn (75 µm × 2 cm, C18, 3 µm, 100 Å; Thermo Scientific) that was connected to an EASY-Spray, PepMap RSLC column (75 µm × 25 cm, C18, 2 µm, 100 Å; Thermo Scientific) with a 5 µm emitter using the autosampler of an EASY-nLC 1000 (Thermo Scientific). Peptides were gradient eluted from the column directly into a Q Exactive mass spectrometer (Thermo Scientific) using a 120 min gradient from 2 % solvent B to 40 % solvent B. Solvent A was 2 % acetonitrile in 0.5 % acetic acid and solvent B was 90 % acetonitrile in 0.5 % acetic acid. High-resolution MS1 spectra were acquired with a resolution of 70 000, an AGC target of 1e6, with a maximum ion time of 120 ms and scan range of 400 to 1500 *m*/*z*. Following each MS1, 20 data-dependent high-resolution HCD MS2 spectra were acquired. All MS2 spectra were collected using the following instrument parameters: resolution of 17 500, AGC target of 5e4, maximum ion time of 250 ms, one microscan, 2 *m*/*z* isolation window, fixed first mass of 150 *m*/*z*, 30 s exclusion list and NCE of 27. The MS/MS spectra were searched against a Uniprot database constructed from *P. mirabilis* HI4320 and *P. mirabilis* ATCC 29906 using Sequest within Proteome Discoverer. Only proteins with two or more high-quality peptide matches were included in the analysis.

RESULTS

Greek classification

Chaperone-usher fimbriae are protein structures located on the surface of Gram-negative bacteria, and are named after the periplasmic chaperone and outer membrane usher proteins that are required for folding and assembly of the fimbria. These fimbriae have been classified into nine groups (α, β, γ_1, γ_2, γ_3, γ_4, κ, π and σ) by evolutionary descent using chaperone sequence, gene order within fimbrial operons and conserved protein domains in the major structural subunits (Nuccio & Bäumler, 2007). Using this method, we provide here the Greek classification for the 17 fimbriae encoded by *P. mirabilis* (Tables 1 and S2). All except Fim14 can be classified into three clades (γ_1, γ_2, π). These three clades all have chaperones of the FGS subfamily (short F_1-G_1 loop; Hung *et al.*, 1996), and major fimbrial subunits with the conserved domains PFAM00419 and COG3539. The majority of the fimbriae encoded by *P. mirabilis* belong to either the γ_1 or the π clade (Table 1). The γ_1 clade operon structure consists at minimum of a major subunit, followed by a chaperone, an usher and a tip adhesin. Eight of the fimbriae encoded by *P. mirabilis* belong to the γ_1 clade (Table 1). The prototypical member of this clade is the type I fimbria of *E. coli*, which is associated with UTI [reviewed by Hannan *et al.* (2012)]. Seven *P. mirabilis* fimbriae belong to the π clade (Table 1). The core genetic organization for operons of this clade consists of a major fimbrial subunit and an usher, followed by a chaperone. The prototypical member of this clade is the uropathogenic *E. coli* P fimbria, which contributes to pyelonephritis [reviewed by Lane & Mobley (2007)]. Fim12 is a member of the γ_2 clade, which is identified by the presence of three chaperones encoded within the operon. The *fim14* operon does not encode a chaperone and therefore cannot be categorized using the Greek classification system.

Table 1. Greek classification of *P. mirabilis* fimbriae

Class	Fimbriae*	Operon organization†
γ_1	UCA, Fim6, Fim7, Fim8, Fim10, ATF, Fim16, Fim17	M, C, U
γ_2	Fim12	M, C, C, C, U
π	MR/P', MR/P, Fim3, Fim5, PMF, PMP, Fim15	M, U, P

*Fimbria 14 not determined – lacks chaperone.

†M, Major subunit; C, chaperone; U, usher; minor subunits and tip adhesins are not shown.

Presence of the 17 fimbriae in sequenced *P. mirabilis* strains

Following the original sequencing of the *P. mirabilis* HI4320 genome (Pearson *et al.*, 2008), the genomes of six new *P. mirabilis* strains [ATCC 29906, BB2000 (Sullivan *et al.*, 2013), WGLW4, WGLW6, C05028 and PR03 (Khalid *et al.*, 2013)] have been sequenced and deposited in GenBank. These strains were obtained from diverse locations: ATCC 29906 is the *P. mirabilis* type strain; BB2000 is a laboratory strain (Belas *et al.*, 1991) that has been the basis of several swarming-motility and strain-identity studies (e.g. Belas *et al.*, 1991, 1998; Gibbs *et al.*, 2008); WGLW4 was isolated from the urine of a human subject; WGLW6 was isolated from the stool of a mouse; C05028 was isolated from the stool of a patient with diarrhoea during an outbreak in Shenzhen, China; and PR03 was found in the blood of a patient with septicaemia in Putrajaya, Malaysia. BLAST was performed to determine the percentage identity of the predicted amino acid sequence of the major structural subunit for all 17 fimbriae in comparison to HI4320 (Tables 2 and S2). Interestingly, 13 of the 17 fimbriae had $\geqslant$99 % identity with HI4320; an additional fimbria was $\geqslant$95 % identical. The remaining three fimbriae (Fim3A, UcaA and Fim6A) had greater diversity in amino acid identity. The only fimbrial major subunit that was not found in all sequenced strains was Fim3A, which was absent from BB2000 and C05028.

Three of these sequenced genomes (HI4320, ATCC 29906 and BB2000) have been annotated, allowing us to examine the organization of the fimbrial operons in these strains. Further genomic comparison showed that all 17 fimbrial operons initially identified in HI4320, including 11 fimbria-associated *mrpJ*-type transcriptional regulators (Pearson & Mobley, 2008), were also present in ATCC 29906. In BB2000, 16 of the operons were present; the *fim3* operon was missing except for an orphaned putative adhesin gene. The location of fimbrial operons in the bacterial chromosome was conserved between HI4320 and BB2000 with the exception of the *uca* operon, which in both strains was found adjacent to phage genes.

Detection of the 17 fimbriae in 1980s *P. mirabilis* isolates

Primers designed to recognize the predicted major structural subunit of each of the 17 fimbriae (the first gene in the operon which encodes a fimbrial structural gene; Table S2) were designed using the genomic sequence from HI4320. We examined 10 *P. mirabilis* isolates obtained from two nursing homes in Baltimore over 30 years ago (Warren *et al.*, 1982). From Fig. 1 and Table 3, it can be seen that the presence of the 17 fimbriae is highly conserved in these strains. Three out of the ten clinical isolates tested positive for all of the fimbriae. The only genes that were not detected in all 10 strains were *fim3A* (detected in 3 of 10) and *fim5A* (6 of 10).

Table 2. Amino acid identity of predicted fimbrial major structural subunits compared to HI4320

Strain	MrpA'	MrpA	Fim3A	UcaA	Fim5A	Fim6A	Fim7A	Fim8A	PmfA	Fim10A	PmpA	Fim12A	AtfA	Fim14A	Fim15A	Fim16A	Fim17A
ATCC 29906	100 %	100 %	75 %	82 %	100 %	99 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	99 %	100 %	100 %
BB2000	100 %	100 %	–	96 %	100 %	51 %	95 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	99 %	100 %
WGLW4	100 %	100 %	100 %	41 %	99 %	98 %	97 %	100 %	99 %	100 %	100 %	100 %	100 %	99 %	99 %	100 %	100 %
WGLW6	100 %	100 %	100 %	95 %	100 %	95 %	99 %	100 %	99 %	100 %	100 %	99 %	99 %	100 %	99 %	100 %	100 %
C05028	100 %	100 %	–	82 %	99 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
PR03	100 %	100 %	76 %	82 %	100 %	90 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	99 %	99 %	100 %

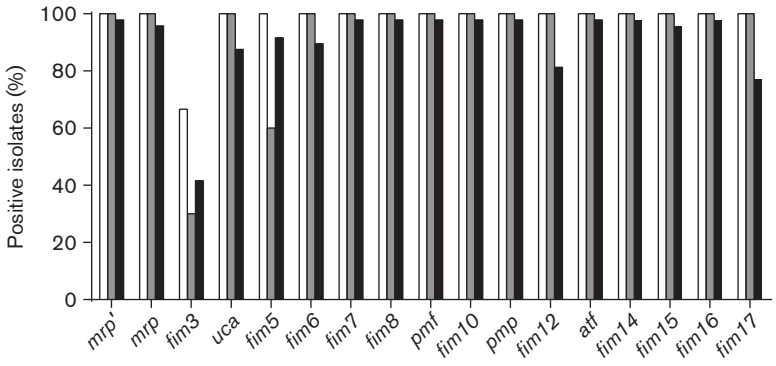

Fig. 1. Conservation of fimbriae encoded by *P. mirabilis*. The percentage of isolates with each fimbria is shown. For the sequenced strains (white bars), fimbriae were identified using BLAST. For the 1980s isolates (grey bars) and hospital isolates (NYU) (black bars), fimbrial genes were detected by PCR.

Conservation of the 17 fimbriae in the NYU collection

Clinical isolates of *Proteus* sp., *Providencia* sp. and *Morganella* sp. [collectively known as tribe *Proteeae* (O'Hara *et al.*, 2000)] were collected from Tisch Hospital at NYULMC from 17 July 2012 to 23 October 2012. A total of 205 strains were collected during this time (166 *P. mirabilis*, 8 *Proteus vulgaris*, 3 *Proteus penneri*, 7 *Providencia stuartii*, 3 *Providencia rettgeri* and 18 *Morganella morganii*). Of the 166 *P. mirabilis* strains, 124 (75 %) were obtained from urine samples, 22 (13 %) from wounds or abscesses, 7 (4 %) from sputum, 5 (3 %) from incision or drainage sites, 2 from the genital tract (1 %) and 6 (4 %) from other sites.

We selected 48 *P. mirabilis* isolates for screening by PCR to determine the presence of the predicted major structural subunit genes for each of the 17 fimbriae. The isolates tested were the first 40 urine isolates plus an additional 8 isolates obtained from diverse anatomical sites (Table S3). Results from the NYU strains were consistent with the findings from the nursing home isolates collected in the 1980s (Fig. 1). The majority of the fimbriae were conserved; that is, 47 out of 48 clinical isolates tested positive for at least 13 of the 17 fimbriae (Fig. 2; Table S3). Sixteen isolates were positive for all 17 fimbriae. The *fim3A* gene was only detected in 20 of 48 (42 %) isolates. Fimbriae that were less conserved in the NYU *P. mirabilis* strains compared with the 1980s isolates were *ucaA* (42/48), *fim6A* (43/48), *fim12A* (39/48) and *fim17A* (37/48). All others were detected in at least 44 of 48 strains (92 %). In contrast to the 1980s collection, the *fim5A* gene was highly conserved (44/48) in the NYU collection. The presence of specific fimbrial genes did not correlate with the anatomical site of *P. mirabilis* isolation (Table S3). The *mrpA* gene, which is generally conserved and required to establish infection in a mouse model of UTI (Bahrani *et al.*, 1994; Jansen *et al.*, 2004), was not detected in two *P. mirabilis* clinical isolates (4 %). Also of note is isolate NYU032 in which 14 fimbrial genes were not detected. Although this isolate was identified as *P. mirabilis* by the clinical laboratory, we further tested this strain to confirm its identity. Primers for *rpoA* (specific for the genus *Proteus*) and *flhD* (specific to *P. mirabilis* based on currently available genome sequences) yielded PCR products with genomic DNA, and this strain swarmed on a hard agar surface (indicative of *Proteus* sp.; data not shown). We also screened one *P. vulgaris* blood isolate and one *M. morganii* urine isolate for the presence of the 17 fimbriae. The *P. vulgaris* isolate was positive for *mrpA*, *ucaA* and *atfA* genes, while none of the fimbrial genes were detected in the *M. morganii* strain.

In order to gauge the genetic diversity of the clinical isolates, we chose five representative strains for further analysis. Four of these strains (NYU009, NYU037, NYU052 and NYU066) were urine isolates collected in different weeks. The fifth isolate, NYU011, was collected the same week as NYU009, but was isolated from an abscess. We sequenced the MrpA- and FlaA (flagellin)-encoding genes for each of these strains and compared the sequences to the available *P. mirabilis* genomes. As expected, the *mrpA* sequence was 100 % identical in all strains examined, with the exception of a single synonymous mutation in NYU011. In contrast, while

Table 3. PCR detection of predicted fimbrial major subunit genes in 1980s isolates

mrpA', *mrpA*, *ucaA*, *fim6A*, *fim7A*, *fim8A*, *pmfA*, *fim10A*, *pmpA*, *fim12A*, *atfA*, *fim14A*, *fim15A*, *fim16A* and *fim17A* were detected in all 10 isolates.

Strain	HI4320	NI114 #800	TA507 #1249	MC118 #1296	HA135 #2537	RU107 #34	MA112 #348	HU119 #798	HA139 #3871	RO106 #23	FE513 #4244
fim3A	+	−	+	−	−	−	+	−	−	+	−
fim5A	+	−	+	−	+	−	+	−	+	+	+

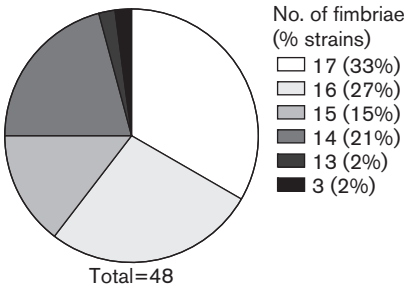

Fig. 2. The majority of fimbriae are highly conserved in recent clinical isolates. The percentage of NYU *P. mirabilis* isolates encoding given numbers of the 17 fimbriae is depicted. A total of 48 isolates were screened.

flaA was conserved at the 5′ and 3′ ends, there was a highly variable region corresponding to nucleotides 531–785 of HI4320 *flaA*. Based on alignment of the sequences, the relatedness of these *flaA* genes was plotted as a phylogenetic tree (Fig. 3). With the exception of NYU037 and NYU052 (which clustered with WGLW4 and HI4320), the *flaA* sequences from the NYU collection did not cluster together; rather, they were distributed among *flaA* from the sequenced *P. mirabilis* genomes.

Expression of 17 fimbriae in HI4320

To determine whether all 17 fimbrial operons are transcribed, qRT-PCR was performed with cDNA derived from HI4320 cultured under different conditions (exponential-phase aerated, stationary aerated, stationary static, pH 6.0, pH 8.0 and colonies on an agar surface). We detected transcript for the major structural genes of all 17 fimbriae (Fig. 4). During exponential-phase aerated culture, *ucaA* was the most abundant transcript, followed by *pmfA* and *fim8A*. The least abundant transcripts were *fim6A* and *fim10A* (mean cycle threshold 30.2 and 31.8, respectively).

To evaluate differential expression of fimbrial genes under specific culture conditions, gene expression was compared to that from exponential-phase aerated culture (Table 4). Experimental conditions were chosen to address an environment that *P. mirabilis* might encounter during infection. The bladder is a reduced-oxygen environment, which was mimicked here by culturing bacteria to stationary phase and/or statically (without shaking). In addition, stationary-phase static culture is a long-established method for increasing fimbriation in different bacterial species including *P. mirabilis* (Old & Duguid, 1970; Old & Adegbola, 1982; Bahrani & Mobley, 1994; Lane *et al.*, 2009). Agar-grown colonies were used to assess bacterial growth on a surface, such as on the bladder epithelium or a urinary catheter. Urine in a healthy individual is normally slightly acidic (pH 5.5–6.5) (Simerville *et al.*, 2005). However, *P. mirabilis* in urine rapidly alkalinizes its environment via urease activity, and so we tested the effect of pH on fimbrial gene expression.

Fimbrial genes were generally induced during stationary-phase culture, and even more so during static culture (Table 4). The most highly induced gene during static culture was *mrpA* (8943-fold mean), with the fold change in five experiments ranging from 70- to 35 108-fold. Fimbrial genes were also induced in colonies, with the exception of *fim7A* and *pmfA*. At pH 8.0, *fim10A* and *fim14A* were induced (2.04-fold and 27-fold, respectively); there was little change in expression for the other genes at pH 6.0 or 8.0.

Multiple fimbrial proteins are produced in a single culture

Given that quantitative PCR detected transcription of all 17 major fimbrial subunits, we were curious to discover which

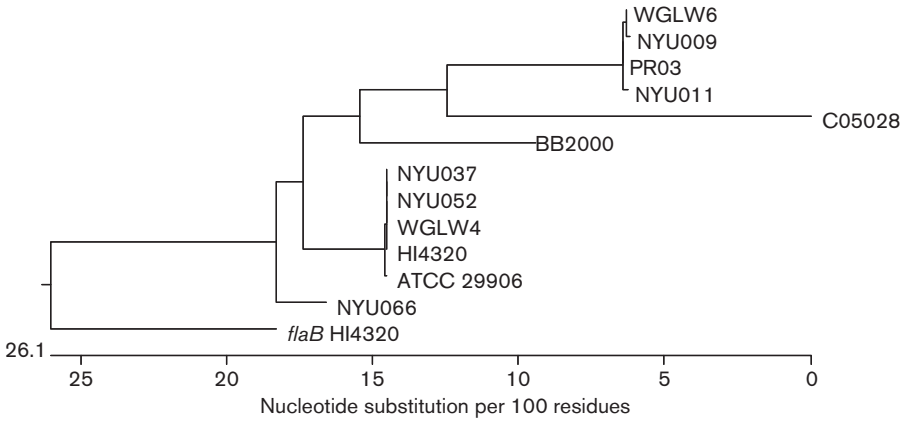

Fig. 3. Phylogenetic tree of *flaA* sequences. The CLUSTAL W algorithm was used to align *flaA* from five NYU clinical isolates and the sequenced *P. mirabilis* genomes. The related *flaB* gene (PMI1619) from HI4320 was included as a reference, and clustered separately from the *flaA* sequences.

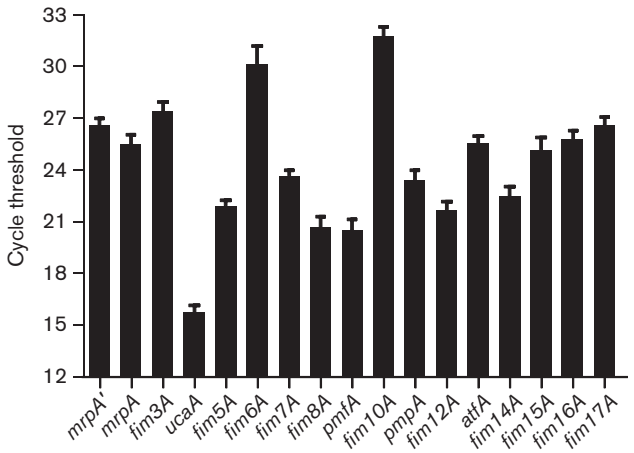

Fig. 4. Expression of fimbrial genes during exponential-phase aerated culture. The cycle threshold of detection for each of the predicted major structural subunit genes is depicted; a lower value indicates more abundant transcript. The data are the result of eight experiments. The error bars represent SEM.

fimbrial proteins are translated in wild-type *P. mirabilis*. To enrich for fimbrial production while limiting the amount of cell lysis, *P. mirabilis* was cultured at 37 °C with aeration for 8 h. Proteins were identified by MS after enrichment for extracellular proteins by first shearing extracellular proteins from the surface and then concentrating the soluble proteins. This enrichment technique has historically been used to identify specific fimbriae produced by *P. mirabilis* (Wray *et al.*, 1986; Bahrani & Mobley, 1993; Massad *et al.*, 1994a; Bijlsma *et al.*, 1995). We detected 232 *P. mirabilis* proteins, the most abundant of which were FlaA (the primary flagellin) and PmfA (the major subunit of the PMF fimbria). The proteins detected included those from the cytoplasm, periplasm and inner membrane, suggesting that some cell lysis occurred during growth and the shearing process; the 50 most abundant proteins detected are listed in Table S4. In total, we detected components from six different fimbriae (Table 5). The PMF fimbria was most abundant, with the major fimbrial subunit (PmfA), tip adhesin (PmfE) and chaperone (PmfD) identified. Fim8 was the second most abundant fimbria found; the major fimbrial subunit (Fim8A), a minor fimbrial subunit (Fim8F) and the tip adhesin (Fim8E) were detected. The remaining fimbriae were of lower abundance, and with the exception of UCA, only the putative major subunit was detected. The peptides detected for each fimbrial protein are listed in Table S5.

DISCUSSION

The initial discovery that *P. mirabilis* encodes 17 chaperone-usher fimbrial operons (Pearson *et al.*, 2008) was surprising because, to our knowledge, no other bacterial species encodes more fimbriae within a single genome. Here we have shown that the chaperone-usher fimbriae encoded by *P. mirabilis* are highly conserved among strains collected from diverse sources. This finding is unusual compared with other bacterial species with large repertoires of fimbrial adhesins: studies with fimbrial genes in *S. enterica* and *E. coli* have shown that the majority of fimbrial genes are highly variable and only a few fimbrial operons are present in all the strains tested (Townsend *et al.*, 2001; Gibbs *et al.*, 2008; Wurpel *et al.*, 2013). Among species that possess multiple fimbriae, the distribution of these fimbriae often correlates with isolation from a particular niche. For example, the P fimbria, which mediates adherence to kidney epithelia, is more prevalent in uropathogenic *E. coli* compared with faecal commensal or pathogenic *E. coli* associated with other disease sites (Källenius *et al.*, 1981). In contrast, we found that fimbrial genes in *P. mirabilis* are highly conserved regardless of location isolated. Not only were these fimbriae conserved when isolated from different parts of the body, but also the fimbrial genes were highly conserved within strains isolated in different parts of the world and within strains collected 30 years apart. The high degree of conservation within these genes indicates that fimbrial genes are important for the life cycle of *P. mirabilis* and have been maintained by evolutionary pressure. An alternative explanation is that *P. mirabilis* is more clonal than *E. coli* or *S. enterica*. However, we do not believe this to be the case: *P. mirabilis* is found in a variety of environments and animal hosts (O'Hara *et al.*, 2000), and our comparison of the major flagellin gene *flaA* shows that some genetic sequences are variable in this species.

We further note that our results for fimbrial conservation in clinical isolates are likely an underestimate for the presence of these genes. We used two primer sets for each gene to increase the likelihood of detecting a gene if it were present, but the possibility remains that the fimbriae are more ubiquitous than we are reporting here. A lack of detection by PCR for specific fimbrial genes in the two clinical isolate collections may be due to variable sequences within the primer binding sites of these fimbrial genes and not due to the lack of their presence. In particular, the Fim3A, UcaA and Fim6A proteins had considerable amino acid variability in the seven sequenced *P. mirabilis* strains. Of the 17 fimbrial operons, only *fim3* was not detected in a majority of isolates. Although we designed primer sets to target conserved regions of the fimbrial genes (by aligning the sequences from the six sequenced genomes), there is not a sufficiently conserved region of *fim3A* that would allow universal detection by PCR. Therefore, this fimbria is likely present in a greater percentage of strains than we reported here. The diversity in UCA is especially interesting since this fimbria has been implicated in adherence to shed human uroepithelial cells (Wray *et al.*, 1986) and as a contributor to virulence in a mouse model of UTI (Pellegrino *et al.*, 2013).

We had anticipated using fimbrial distribution to predict which proteins might be associated with colonization of particular niches. Instead, it may be possible that while

Table 4. Fold change in expression of fimbrial genes during different culture conditions compared to exponential-phase aerated culture

P is the probability result of a two-tailed *t*-test (values below 0.050 are shown in bold).

Condition*	Value	*mrpA'*	*mrpA*	*fim3A*	*ucaA*	*fim5A*	*fim6A*	*fim7A*	*fim8A*	*pmfA*	*fim10A*	*pmpA*	*fim12A*	*atfA*	*fim14A*	*fim15A*	*fim16A*	*fim17A*
Stationary static	Mean	205	8943	145	23.47	16.99	2992	7.53	4.28	37.41	21.06	3.646	73.44	201.3	5.66	203	120.7	428.9
	SD	213.3	14946	170.7	17.62	13.25	5591	7.71	6.19	31.51	26.11	2.39	61.86	126.9	6.87	282.7	102.3	588.5
	P	0.099	0.252	0.132	**0.046**	0.054	0.298	0.131	0.302	0.061	0.161	0.069	0.059	**0.024**	0.204	0.185	0.059	0.179
Stationary aerated	Mean	32.36	80.42	50.66	5.14	5.48	10.53	1.01	50.84	4.11	27.87	5.69	26.74	21.44	18.55	91.33	8.57	130.6
	SD	14.29	39.01	31.50	2.65	3.86	6.09	0.603	34.85	2.56	17.86	3.29	16.27	11.98	14.99	65.02	3.52	48.34
	P	**0.022**	**0.027**	0.051	0.052	0.103	0.052	0.988	0.065	0.093	0.057	0.065	0.051	**0.042**	0.101	0.069	**0.023**	**0.013**
Plate	Mean	17.35	46.92	15.21	7.21	4.58	12.28	2.29	111.1	0.876	4.56	131.9	19.28	9.40	19.15	177.6	5.10	69.31
	SD	20.05	60.94	11.63	6.76	2.67	7.62	1.44	93.59	0.285	1.60	129.3	11.96	5.91	18.93	264.7	5.57	74.41
	P	0.293	0.322	0.169	0.253	0.146	0.124	0.261	0.179	0.528	0.061	0.222	0.118	0.133	0.239	0.367	0.331	0.253
pH 6	Mean	1.67	0.847	0.574	0.485	0.737	0.450	0.554	0.504	1.02	0.427	3.05	1.26	3.96	4.88	4.33	1.00	3.07
	SD	1.22	0.596	0.417	0.256	0.499	0.300	0.306	0.354	1.17	0.215	1.93	0.854	2.67	2.62	3.57	0.654	2.03
	P	0.444	0.700	0.219	0.074	0.457	0.087	0.127	0.136	0.975	**0.044**	0.209	0.646	0.195	0.124	0.248	0.997	0.220
pH 8	Mean	2.55	1.51	2.74	1.07	1.79	1.55	1.40	1.02	2.69	2.04	6.01	3.53	9.28	26.85	1.93	1.05	6.85
	SD	1.50	0.266	1.16	0.278	0.942	0.537	0.464	0.321	0.872	0.521	7.43	3.00	7.09	16.99	1.26	0.925	4.39
	P	0.215	0.081	0.121	0.694	0.286	0.216	0.278	0.915	0.079	0.074	0.363	0.282	0.180	0.119	0.329	0.934	0.147

*For stationary static, $n=5$ independent experiments; for stationary aerated, $n=4$; for all other experiments, $n=3$.

Table 5. Detection of fimbrial proteins using MS

Proteins from concentrated *P. mirabilis* shear preparations were identified by searching detected peptides against a *P. mirabilis* database. Measurements of percent age coverage, the number of unique peptides detected and the number of total peptides allows an approximation of the relative quantity of protein present.

Protein ID	ORF	Gene	Annotation	Coverage (%)	Unique peptides	Total peptides
PMF						
Q04681	PMI1877	*pmfA*	Major fimbrial subunit	81.0	12	573
P53522	PMI1880	*pmfE*	Putative tip adhesin	52.7	12	53
P53520	PMI1879	*pmfD*	Fimbrial chaperone protein	9.1	2	2
Fimbria 8						
B4EXA4	PMI1469	*fim8A*	Fimbrial subunit	61.3	8	75
B4EXA0	PMI1465	*fim8E*	Putative fimbrial adhesin	25.6	6	6
B4EX99	PMI1464	*fim8F*	Fimbrial subunit	12.4	2	2
MR/P						
Q03011	PMI0263	*mrpA*	Major MR/P fimbrial protein	54.9	6	30
UCA						
B4EV68	PMI0536	*ucaA*	Major UCA fimbrial subunit	42.0	5	21
B4EV67	PMI0535	*ucaB*	Fimbrial chaperone	15.8	3	3
B4EV66	PMI0534	*ucaC*	Fimbrial usher protein	7.4	5	5
Fimbria 14						
B4F012	PMI3002	*fim14A*	Putative fimbrial protein	48.3	8	15
ATF						
B4EYH3	PMI2728	*atfA*	Major fimbrial subunit	22.0	3	14

most of these fimbriae are ubiquitous in *P. mirabilis*, amino acid variations within a given fimbria confer an advantage in specific environments. This phenomenon has already been observed for the type I fimbria, which is present in the vast majority of *E. coli* isolates (Mobley *et al.*, 2009). Specific amino acid sequences of the FimH adhesin are more likely to be found in uropathogenic *E. coli* compared with faecal *E. coli*, and these variations alter binding characteristics (Sokurenko *et al.*, 1995, 1998). These binding properties may be tied to the need for uropathogens to adhere in the face of urine flow, or to mediate adherence to specific displays of mannose (e.g. monomannose vs trimannose) (Sokurenko *et al.*, 1997; Schembri & Klemm, 2001). In our current study, we used a PCR assay to look for the presence of the predicted major structural subunit genes of these fimbriae, because we expected a large proportion of fimbrial operons would be completely absent from many isolates. However, if fimbrial presence is conserved while binding affinity varies, we would expect to find variation in the tip adhesins for these fimbriae.

We detected transcripts corresponding to the predicted major structural subunit of all 17 fimbriae, as well as proteins from six predicted fimbrial major subunits. However, we do not yet know whether the remaining fimbrial transcripts are translated, nor do we know whether all of the detected subunits are assembled into mature fimbriae at the cell surface. In addition to the 17 fimbrial operons described here, the *P. mirabilis* HI4320 genome contains 13 additional putative fimbrial structural genes that are not organized with genes encoding chaperones or ushers (Pearson *et al.*, 2008); however, we did not examine these orphans in this study. Our finding of fimbrial gene upregulation during static or stationary-phase culture is consistent with previous reports on fimbrial production by *P. mirabilis* and other species (Old & Duguid, 1970; Blomfield *et al.*, 1991; Lane *et al.*, 2009). In particular, MR/P fimbriae are assembled in greater numbers during controlled oxygen restriction, as well as during infection of mice (Lane *et al.*, 2009). Expression of *fim10A* and *fim14A* was induced under alkaline conditions, which would occur in the urinary tract due to urease activity during UTI. Consistent with this idea, another *P. mirabilis* virulence factor, the *Proteus* toxic agglutinin, has previously been shown to have increased activity at basic pH (Alamuri & Mobley, 2008).

Our results show for the first time that a single population of *P. mirabilis* can produce six major fimbrial subunits that likely results in the assembly of six distinct fimbriae on the cell surface. We also detected proteins from Fim8 and Fim14 for the first time. Others have previously reported detection of MR/P, PMF, UCA, ATF and PMP protein (Adegbola *et al.*, 1983; Wray *et al.*, 1986; Bahrani *et al.*, 1993, 1994; Massad *et al.*, 1994a; Bijlsma *et al.*, 1995; Cook *et al.*, 1995), although not necessarily all at the same time. Electron microscopy has shown that a single *P. mirabilis* bacterium can express at least two distinct types of fimbriae, which correspond to two unique haemagglutination patterns (Old & Adegbola, 1982; Adegbola *et al.*, 1983). Analysis of MR/P and ATF revealed that both proteins are produced in a single culture grown statically for 48 h (Zunino *et al.*, 2007). Our data do not distinguish between a

single bacterium expressing up to six distinct fimbriae and population-wide variation in gene expression resulting in different bacteria presenting different fimbriae on their surface. Because tools (e.g. antibodies) are developed to study these proteins, this question may be addressed.

The presence of Fim14A is particularly interesting, as the *fim14* operon lacks a chaperone and its usher in HI4320 is a pseudogene with an internal frameshift. Notably, the usher gene appears intact in the annotated ATCC 29906 and BB2000 genomes, but the chaperone is entirely absent. A chaperone and usher are essential for exporting fimbrial subunits to the extracellular space, so it is possible that Fim14A is not exported, but instead is cytoplasmic protein that we detected due to cell lysis. A more intriguing possibility is that Fim14A is exported to the bacterial surface via interaction with another fimbrial chaperone/usher or with an orphan chaperone/usher. It has been shown that in the absence of the native chaperone and usher, *E. coli* type 1 and F1C fimbriae can be functionally assembled by a heterologous chaperone and usher pair (Klemm *et al.*, 1994, 1995). Although we are unaware of any instances in which a fimbria is natively assembled by a heterologous chaperone and usher pair, the identification of Fim14A as a putatively exported protein and *fim14B* as a gene important for pathogenesis (Himpsl *et al.*, 2008) suggests that Fim14 is assembled into a functional fimbria.

In summary, we have documented the surprisingly high degree of conservation of the 17 chaperone-usher fimbrial operons encoded by *P. mirabilis*. It would be interesting to further investigate conservation of other, non-fimbrial genes encoded by diverse *P. mirabilis* isolates to determine the heterogeneity of virulence factors. For example, genes involved in individual strain self-recognition are not conserved when the two sequenced strains HI4320 and BB2000 are compared (Sullivan *et al.*, 2013). Future studies will dissect the roles of specific fimbriae in colonization and disease toward the goal of designing better treatments or vaccines against *P. mirabilis*-mediated UTI.

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REFERENCES

Adegbola, R. A., Old, D. C. & Senior, B. W. (1983). The adhesins and fimbriae of *Proteus mirabilis* strains associated with high and low affinity for the urinary tract. *J Med Microbiol* **16**, 427–431.

Alamuri, P. & Mobley, H. L. (2008). A novel autotransporter of uropathogenic *Proteus mirabilis* is both a cytotoxin and an agglutinin. *Mol Microbiol* **68**, 997–1017.

Alamuri, P., Löwer, M., Hiss, J. A., Himpsl, S. D., Schneider, G. & Mobley, H. L. (2010). Adhesion, invasion, and agglutination mediated by two trimeric autotransporters in the human uropathogen *Proteus mirabilis*. *Infect Immun* **78**, 4882–4894.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Armbruster, C. E. & Mobley, H. L. T. (2012). Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. *Nat Rev Microbiol* **10**, 743–754.

Bahrani, F. K. & Mobley, H. L. (1993). *Proteus mirabilis* MR/P fimbriae: molecular cloning, expression, and nucleotide sequence of the major fimbrial subunit gene. *J Bacteriol* **175**, 457–464.

Bahrani, F. K. & Mobley, H. L. T. (1994). *Proteus mirabilis* MR/P fimbrial operon: genetic organization, nucleotide sequence, and conditions for expression. *J Bacteriol* **176**, 3412–3419.

Bahrani, F. K., Cook, S., Hull, R. A., Massad, G. & Mobley, H. L. (1993). *Proteus mirabilis* fimbriae: N-terminal amino acid sequence of a major fimbrial subunit and nucleotide sequences of the genes from two strains. *Infect Immun* **61**, 884–891.

Bahrani, F. K., Massad, G., Lockatell, C. V., Johnson, D. E., Russell, R. G., Warren, J. W. & Mobley, H. L. (1994). Construction of an MR/P fimbrial mutant of *Proteus mirabilis*: role in virulence in a mouse model of ascending urinary tract infection. *Infect Immun* **62**, 3363–3371.

Belas, R., Erskine, D. & Flaherty, D. (1991). Transposon mutagenesis in *Proteus mirabilis*. *J Bacteriol* **173**, 6289–6293.

Belas, R., Schneider, R. & Melch, M. (1998). Characterization of *Proteus mirabilis* precocious swarming mutants: identification of *rsbA*, encoding a regulator of swarming behavior. *J Bacteriol* **180**, 6126–6139.

Bijlsma, I. G. W., van Dijk, L., Kusters, J. G. & Gaastra, W. (1995). Nucleotide sequences of two fimbrial major subunit genes, *pmpA* and *ucaA*, from canine-uropathogenic *Proteus mirabilis* strains. *Microbiology* **141**, 1349–1357.

Blomfield, I. C., McClain, M. S., Princ, J. A., Calie, P. J. & Eisenstein, B. I. (1991). Type 1 fimbriation and *fimE* mutants of *Escherichia coli* K-12. *J Bacteriol* **173**, 5298–5307.

Chaudhuri, R. R., Loman, N. J., Snyder, L. A. S., Bailey, C. M., Stekel, D. J. & Pallen, M. J. (2008). *x*BASE2: a comprehensive resource for comparative bacterial genomics. *Nucleic Acids Res* **36** (Database), D543–D546.

Cook, S. W., Mody, N., Valle, J. & Hull, R. (1995). Molecular cloning of *Proteus mirabilis* uroepithelial cell adherence (*uca*) genes. *Infect Immun* **63**, 2082–2086.

Cotto-Rios, X. M., Békés, M., Chapman, J., Ueberheide, B. & Huang, T. T. (2012). Deubiquitinases as a signaling target of oxidative stress. *Cell Reports* **2**, 1475–1484.

DeFrances, C. J., Lucas, C. A., Buie, V. C. & Golosinskiy, A. (2008). *2006 National Hospital Discharge Survey*; National Health Statistics Reports, no. 5. Hyattsville, MD: National Center for Health Statistics..

Foxman, B. (2003). Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Dis Mon* **49**, 53–70.

Gaastra, W., van Oosterom, R. A., Pieters, E. W., Bergmans, H. E., van Dijk, L., Agnes, A. & ter Huurne, H. M. (1996). Isolation and characterisation of dog uropathogenic *Proteus mirabilis* strains. *Vet Microbiol* **48**, 57–71.

Gibbs, K. A., Urbanowski, M. L. & Greenberg, E. P. (2008). Genetic determinants of self identity and social recognition in bacteria. *Science* **321**, 256–259.

Griffith, D. P., Musher, D. M. & Itin, C. (1976). Urease. The primary cause of infection-induced urinary stones. *Invest Urol* **13**, 346–350.

Hannan, T. J., Totsika, M., Mansfield, K. J., Moore, K. H., Schembri, M. A. & Hultgren, S. J. (2012). Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection. *FEMS Microbiol Rev* **36**, 616–648.

Himpsl, S. D., Lockatell, C. V., Hebel, J. R., Johnson, D. E. & Mobley, H. L. (2008). Identification of virulence determinants in uropathogenic *Proteus mirabilis* using signature-tagged mutagenesis. *J Med Microbiol* **57**, 1068–1078.

Hung, D. L., Knight, S. D., Woods, R. M., Pinkner, J. S. & Hultgren, S. J. (1996). Molecular basis of two subfamilies of immunoglobulin-like chaperones. *EMBO J* **15**, 3792–3805.

Jansen, A. M., Lockatell, V., Johnson, D. E. & Mobley, H. L. (2004). Mannose-resistant *Proteus*-like fimbriae are produced by most *Proteus mirabilis* strains infecting the urinary tract, dictate the in vivo localization of bacteria, and contribute to biofilm formation. *Infect Immun* **72**, 7294–7305.

Källenius, G., Svenson, S. B., Hultberg, H., Möllby, R., Helin, I., Cedergren, B. & Winberg, J. (1981). Occurrence of P-fimbriated *Escherichia* coli in urinary tract infections. *Lancet* **318**, 1369–1372.

Khalid, M. I. H., Teh, L. K., Lee, L. S., Zakaria, Z. A. & Salleh, M. Z. (2013). Genome sequence of *Proteus mirabilis* strain PR03, isolated from a local hospital in Malaysia. *Genome Announc* **1**, e00327-13.

Klemm, P., Christiansen, G., Kreft, B., Marre, R. & Bergmans, H. (1994). Reciprocal exchange of minor components of type 1 and F1C fimbriae results in hybrid organelles with changed receptor specificities. *J Bacteriol* **176**, 2227–2234.

Klemm, P., Jørgensen, B. J., Kreft, B. & Christiansen, G. (1995). The export systems of type 1 and F1C fimbriae are interchangeable but work in parental pairs. *J Bacteriol* **177**, 621–627.

Klevens, R. M., Edwards, J. R., Richards, C. L., Jr, Horan, T. C., Gaynes, R. P., Pollock, D. A. & Cardo, D. M. (2007). Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Health Rep* **122**, 160–166.

Lane, M. C. & Mobley, H. L. (2007). Role of P-fimbrial-mediated adherence in pyelonephritis and persistence of uropathogenic *Escherichia coli* (UPEC) in the mammalian kidney. *Kidney Int* **72**, 19–25.

Lane, M. C., Li, X., Pearson, M. M., Simms, A. N. & Mobley, H. L. (2009). Oxygen-limiting conditions enrich for fimbriate cells of uropathogenic *Proteus mirabilis* and *Escherichia coli*. *J Bacteriol* **191**, 1382–1392.

Litwin, M. S. & Saigal, C. S. (2007). *Urologic Diseases in America*. Washington, D.C.: Government Printing Office.

Livak, K. J. & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* **25**, 402–408.

Massad, G., Bahrani, F. K. & Mobley, H. L. (1994a). *Proteus mirabilis* fimbriae: identification, isolation, and characterization of a new ambient-temperature fimbria. *Infect Immun* **62**, 1989–1994.

Massad, G., Lockatell, C. V., Johnson, D. E. & Mobley, H. L. (1994b). *Proteus mirabilis* fimbriae: construction of an isogenic *pmfA* mutant and analysis of virulence in a CBA mouse model of ascending urinary tract infection. *Infect Immun* **62**, 536–542.

Massad, G., Fulkerson, J. F., Jr, Watson, D. C. & Mobley, H. L. (1996). *Proteus mirabilis* ambient-temperature fimbriae: cloning and nucleotide sequence of the *aft* gene cluster. *Infect Immun* **64**, 4390–4395.

Mobley, H. L., Island, M. D. & Hausinger, R. P. (1995). Molecular biology of microbial ureases. *Microbiol Rev* **59**, 451–480.

Mobley, H. L. T., Donnenberg, M. S. & Hagan, E. C. (2009). Uropathogenic *Escherichia coli*. In *EcoSal – Escherichia coli and Salmonella: Cellular and Molecular Biology*. Edited by A. Böck & R. Curtiss, III, J. B. Kaper, P. D. Karp, F. C. Neidhardt, T. Nyström, J. M. Slauch, C. L. Squires & D. Ussery. Washington, D.C.: American Society for Microbiology.

Nielubowicz, G. R. & Mobley, H. L. (2010). Host-pathogen interactions in urinary tract infection. *Nat Rev Urol* **7**, 430–441.

Nuccio, S. P. & Bäumler, A. J. (2007). Evolution of the chaperone/usher assembly pathway: fimbrial classification goes Greek. *Microbiol Mol Biol Rev* **71**, 551–575.

O'Hara, C. M., Brenner, F. W. & Miller, J. M. (2000). Classification, identification, and clinical significance of *Proteus, Providencia*, and *Morganella*. *Clin Microbiol Rev* **13**, 534–546.

Old, D. C. & Adegbola, R. A. (1982). Haemagglutinins and fimbriae of *Morganella, Proteus* and *Providencia*. *J Med Microbiol* **15**, 551–564.

Old, D. C. & Duguid, J. P. (1970). Selective outgrowth of fimbriate bacteria in static liquid medium. *J Bacteriol* **103**, 447–456.

Pearson, M. M. & Mobley, H. L. (2008). Repression of motility during fimbrial expression: identification of 14 *mrpJ* gene paralogues in *Proteus mirabilis*. *Mol Microbiol* **69**, 548–558.

Pearson, M. M., Sebaihia, M., Churcher, C., Quail, M. A., Seshasayee, A. S., Luscombe, N. M., Abdellah, Z., Arrosmith, C., Atkin, B. & other authors (2008). Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. *J Bacteriol* **190**, 4027–4037.

Pellegrino, R., Scavone, P., Umpiérrez, A., Maskell, D. J. & Zunino, P. (2013). *Proteus mirabilis* uroepithelial cell adhesin (UCA) fimbria plays a role in the colonization of the urinary tract. *Pathog Dis* **67**, 104–107.

Roberts, J. A., Fussell, E. N. & Kaack, M. B. (1990). Bacterial adherence to urethral catheters. *J Urol* **144**, 264–269.

Schembri, M. A. & Klemm, P. (2001). Biofilm formation in a hydrodynamic environment by novel *fimh* variants and ramifications for virulence. *Infect Immun* **69**, 1322–1328.

Simerville, J. A., Maxted, W. C. & Pahira, J. J. (2005). Urinalysis: a comprehensive review. *Am Fam Physician* **71**, 1153–1162.

Sokurenko, E. V., Courtney, H. S., Maslow, J., Siitonen, A. & Hasty, D. L. (1995). Quantitative differences in adhesiveness of type 1 fimbriated *Escherichia coli* due to structural differences in *fimH* genes. *J Bacteriol* **177**, 3680–3686.

Sokurenko, E. V., Chesnokova, V., Doyle, R. J. & Hasty, D. L. (1997). Diversity of the *Escherichia coli* type 1 fimbrial lectin. Differential binding to mannosides and uroepithelial cells. *J Biol Chem* **272**, 17880–17886.

Sokurenko, E. V., Chesnokova, V., Dykhuizen, D. E., Ofek, I., Wu, X. R., Krogfelt, K. A., Struve, C., Schembri, M. A. & Hasty, D. L. (1998). Pathogenic adaptation of *Escherichia coli* by natural variation of the FimH adhesin. *Proc Natl Acad Sci U S A* **95**, 8922–8926.

Spurbeck, R. R., Stapleton, A. E., Johnson, J. R., Walk, S. T., Hooton, T. M. & Mobley, H. L. T. (2011). Fimbrial profiles predict virulence of uropathogenic *Escherichia coli* strains: contribution of Ygi and Yad fimbriae. *Infect Immun* **79**, 4753–4763.

Sullivan, N. L., Septer, A. N., Fields, A. T., Wenren, L. M. & Gibbs, K. A. (2013). The complete genome sequence of *Proteus mirabilis* strain BB2000 reveals differences from the *P. mirabilis* reference strain. *Genome Announc* **1**, e00024-13.

Tambyah, P. A. (2004). Catheter-associated urinary tract infections: diagnosis and prophylaxis. *Int J Antimicrob Agents* **24** (Suppl. 1), S44–S48.

Tambyah, P. A. & Maki, D. G. (2000). Catheter-associated urinary tract infection is rarely symptomatic: a prospective study of 1,497 catheterized patients. *Arch Intern Med* **160**, 678–682.

Tanabe, M. & Kanehisa, M. (2012). Using the KEGG database resource. *Curr Protoc Bioinformatics* **38**, 1.12.1–1.12.43.

Tolson, D. L., Barrigar, D. L., McLean, R. J. & Altman, E. (1995). Expression of a nonagglutinating fimbria by *Proteus mirabilis*. *Infect Immun* **63**, 1127–1129.

Townsend, S. M., Kramer, N. E., Edwards, R., Baker, S., Hamlin, N., Simmonds, M., Stevens, K., Maloy, S., Parkhill, J. & other authors (2001). *Salmonella enterica* serovar Typhi possesses a unique repertoire of fimbrial gene sequences. *Infect Immun* **69**, 2894–2901.

Warren, J. W., Tenney, J. H., Hoopes, J. M., Muncie, H. L. & Anthony, W. C. (1982). A prospective microbiologic study of bacteriuria in patients with chronic indwelling urethral catheters. *J Infect Dis* **146**, 719–723.

Wray, S. K., Hull, S. I., Cook, R. G., Barrish, J. & Hull, R. A. (1986). Identification and characterization of a uroepithelial cell adhesin from a uropathogenic isolate of *Proteus mirabilis*. *Infect Immun* **54**, 43–49.

Wurpel, D. J., Beatson, S. A., Totsika, M., Petty, N. K. & Schembri, M. A. (2013). Chaperone-usher fimbriae of *Escherichia coli*. *PLoS ONE* **8**, e52835.

Zunino, P., Geymonat, L., Allen, A. G., Legnani-Fajardo, C. & Maskell, D. J. (2000). Virulence of a *Proteus mirabilis* ATF isogenic mutant is not impaired in a mouse model of ascending urinary tract infection. *FEMS Immunol Med Microbiol* **29**, 137–143.

Zunino, P., Sosa, V., Allen, A. G., Preston, A., Schlapp, G. & Maskell, D. J. (2003). *Proteus mirabilis* fimbriae (PMF) are important for both bladder and kidney colonization in mice. *Microbiology* **149**, 3231–3237.

Zunino, P., Sosa, V., Schlapp, G., Allen, A. G., Preston, A. & Maskell, D. J. (2007). Mannose-resistant *Proteus*-like and *P. mirabilis* fimbriae have specific and additive roles in *P. mirabilis* urinary tract infections. *FEMS Immunol Med Microbiol* **51**, 125–133.