Supporting Information

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SI Text

Strains and Cloning. UTI89 has been described in ref. 1. Deletion of *fimH* and integration of mutant *fimH* alleles was performed using the Red recombinase system described by Datsenko and Wanner (2), and Murphy and Campellone (3). C3H/HeN mice were obtained from the National Cancer Institute and Harlan. All experiments involving mice were performed using protocols approved by the animal studies committee of Washington University.

Reagents. Conjugated BSA (BSA-mannose and BSA-mannotriose) were kindly provided by MedImmune. Guinea pig red blood cells were purchased from Colorado Serum. Enzymes were purchased from New England Biolabs, Invitrogen, and Fisher.

DNA Sequencing. The *fimH* and *fimC* genes were separately amplified in PCR reactions using colonies or purified genomic DNA as template. The primers used to amplify *fimH* were uti8+4913338 and uti8−4915222 (Table S4). The primers used to amplify *fimC* were uti8+4908948 and uti8−4910744. PCRs contained 1× PCR buffer (Invitrogen) supplemented with 2.5 mM $MgCl_2$, 1.4 M betaine, 1.3% DMSO, and 200 μM dNTPs, 5 ng template, 12.5 pmol of each primer, and 1 unit Taq polymerase (Invitrogen). Reactions were held at 95 °C for 5 min, cycled 35 times between 95 °C (1 min), 55 °C (1 min), and 72 °C (3 min), and finished at 72 °C for 10 min. Products were purified (PerfectPrep, Eppendorf) and submitted for capillary sequencing with an ABI 3730xl instrument (SeqWright, Inc.). Sequencing primers included the PCR primers as well as uti8+4913927 for *fimH* and uti8−4910078 for *fimC*. Base calling and assembly was done using Phred and Phrap (4, 5).

Sequence Manipulation. Sequences were aligned using CLUSTALW (6) with default parameters, and trimmed to the start and stop codons of *fimH* and *fimC* as annotated in the UTI89 genome sequence (7). Identical sequences from multiple strains were filtered so that each sequence was represented only once. GENECONV (8) and GARD (9) were used with default parameters to detect recombination. The AICc criterion was used for GARD. Maximum likelihood trees were inferred using PHYML (10), with 100 bootstrap replicates.

Analysis of Positive Selection. PAML version 4 (11) was used for all positive selection analyses. The following models were run on the entire set of *fimH* and *fimC* sequences: M1, M2, M7, and M8. Subtrees of *fimH* were identified based on visual inspection and previous studies of FimH phylogenetic trees (12). Previous analysis of *fimH* sequences identified two major groups based on amino acids coded by positions 70 and 78. This was recapitulated in our analysis by the division between subtree 1 from subtrees 2 and 3. Subtree 2 was identified because these *fimH* sequences were closer to each other than to any in subtree 3 and this division had high bootstrap support (99/100 replicates). Subtrees of *fimC* were more difficult to identify, and less clearly defined than the *fimH* subtrees. Subtrees of *fimC* were therefore chosen to give a similar number of subtrees and similar numbers of *fimC* alleles within each subtree as found for *fimH*. To control against possible bias in *fimC* subtree identification, additional positive selection analysis was performed on the following subdivisions of the *fimC* sequences: (*i*) all sequences from urine isolates versus those from fecal isolates; and (*ii*) all *fimC* sequences whose corresponding *fimH* sequence fell in subtree 1 (or 2 or 3) of the *fimH* phylogeny (i.e., imposing the *fimH* phylogeny and subtree divisions upon the *fimC* sequences). All of these tests gave the same result of no detectable positive selection in *fimC*.

The bsA model was run with foreground branches as specified in Fig. 1 and Fig. S1. Additional site models were run using only sequences within each subtree; for these, additional maximum likelihood phylogenetic trees were inferred using PHYML before running the M1, M2, M7, and M8 models again. Likelihood ratio tests (LRTs) were done assuming a X^2 distribution, using twice the difference in log likelihoods as X^2 and the parameter difference as reported by PAML as the degrees of freedom. A Bonferroni correction was applied for each set of comparisons; this was 4 for the whole tree and bsA models, 5 for the *fimH* subtree analyses, and 4 for the *fimC* subtree analyses. Corrected *P* values <0.05 were considered significant.

Site-Directed Mutagenesis of *fimH*. The *fimH* gene was amplified by PCR (all PCR for cloning used the same recipe described above under DNA Sequencing) from UTI89 genomic DNA with primers uti8+4913338 and uti8−4914820 and cloned into pCR4-TOPO (Invitrogen), resulting in plasmid pSLC2-12-fimH-TOPO-1. The kanamycin resistance cassette from plasmid pKD4 was amplified with primers *Mlu*I-*Kan*L and *Asc*I-*Kan*R, cut with *Mlu*I and *Asc*I, and cloned into the *Mlu*I site of pSLC2-12-fimH-TOPO-1. A clone with the kanamycin resistance cassette transcribed in the same direction as *fimH* was isolated based on restriction digest and PCR assays and named pSLC2-16-2. The 2.4-kb fragment containing *fimH* and the kanamycin resistance gene was cut out from pSLC2-16-2 by digestion with *Not*I and *Spe*I, blunted with the Klenow fragment of *E. coli* DNA Polymerase I, and ligated to the 2.4-kb fragment of pUC19 resulting from complete digestion with *Pvu*II, giving plasmid pSLC2-24-3.

Site-directed mutagenesis of *fimH* was done by PCR on pSLC2–24-3 using primers uti8+4913338, uti8−4915222, and sew primers (Table S4). Two PCR products resulting from amplification of plasmid pSLC2-24-3 (or a mutated derivative) with (*i*) a sew-R primer and uti8+4913338 and (*ii*) a sew-L primer and uti8−4915222 were purified with the QiaQuick PCR purification kit and eluted in 50 μL of the supplied EB buffer. One microliter of each of these purified products was added as template to a final PCR using primers uti8+4913338 and uti8−4915222, yielding a 2.9-kb product. This 2.9-kb fragment was digested with *Mlu*I and *Pvu*II and cloned into pSLC2-24-3 that had been fully digested with *Mlu*I and partially digested with *Pvu*II (cut within the *fimH* gene but not the kanamycin-resistance gene) to yield a mutant derivative of pSLC2-24-3.

Construction of Chromosomal Mutant *fimH* Strains. The Red recombinase system was used as described in refs. 2 and 3 to replace the chromosomal *fimH* gene in UTI89 with a kanamycin resistance cassette from the pKD4 plasmid (2), using a linear PCR fragment made with primers uti8+4913515_fimH-pKD4-left and uti8−4914747_fimH-pKD4-right, resulting in strain SLC2–12-1. The kanamycin resistance cassette was removed by transient expression of the Flp recombinase from plasmid pCP20 (13), resulting in strain SLC2-14-1. The pKM208 plasmid (3) was transformed into SLC2-14-1, yielding strain SLC2-17-fimH. Strain SLC2-17-fimH was made competent per the protocol described in Murphy and Campellone (3) and transformed with the 2.4-kb fragment that resulted from the digestion of pSLC2-24-1 (or a derivative plasmid containing a mutated *fimH* allele) with *Eco*RI and *Bsa*I, and integrants were isolated by plating on

LB-kan. All manipulations were verified to have yielded the correct size product when amplified with PCR primers uti8+4913338 and uti8−4915222. Chromosomal integration junctions and the entire *fimH* gene sequence were verified by sequencing for all *fimH* mutant strains.

Construction of Phase-Locked ON Mutants. This was done as described in ref. 14, using primers FimBE KO#1 and FimBE KO #2, except the template plasmid used was pKD3 (2), resulting in a chloramphenicol-resistant strain.

Type 1 Phase Assay. This assay was modified from Roesch and Blomfield (15) and Struve and Krogfelt (16). Primers used were uti8-phaseL and uti8-phaseR. A 50-μL PCR was run using 1× PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, 200 μM dNTPs (each), 10 pmol of each primer, and 1 unit Taq polymerase. One microliter of bacterial culture was used as template. The reaction was heated to 95 °C for 5 min then cycled 35 times between 95 °C (1 min), 55 °C (1 min), and 72 °C (1 min). Ten microliters of this reaction was mixed with 2 μL of 10× *Hin*fI buffer and 1 unit *Hin*fI restriction enzyme in a 20-μL reaction, incubated at 37 °C for 1 h, and analyzed on a 2% agarose gel.

Type 1-Inducing Cultures. A single colony on solid media was inoculated into 10 mL Luria–Bertani (LB) media (Fisher Scientific) in a 125-mL flask and incubated without shaking at 37 °C for 18–24 h. Ten microliters of this culture was inoculated into 10 mL fresh LB media in a second 125-mL flask and incubated without shaking at 37 °C for 18–24 h.

Immunoblotting. Bacteria grown under type 1 inducing conditions were pelleted (4,000 × *g* for 10 min at 4 °C) and resuspended in PBS to a final OD_{600} of 1.0. One milliliter of this cell suspension was pelleted and resuspended in 100 μL of 4× SDS loading buffer and stored at −20 °C. Before electrophoresis, samples were thawed, mixed thoroughly with 3 μL of 1 M HCl, heated to 95 °C for 5 min, neutralized with 3 μL of 1 M NaOH, and centrifuged at 14,000 × *g* for 2 min. Ten microliters of the resulting supernatant was loaded onto a 12% SDS/PAGE gel, transferred to a nitrocellulose membrane, blotted with a polyclonal rabbit anti-FimA or anti-FimCH sera (1:5,000 dilution), blotted with a monoclonal HRP-conjugated goat anti-rabbit IgG antibody (1:10,000 dilution, Pierce), and detected with the SuperSignal Pico (Pierce) luminescence substrate.

Electron Microscopy. Bacteria were grown under type 1 inducing conditions then prepared for microscopy as described in ref. 17. A semiquantitative scale (from 1 to 10) for three parameters was used to compare pili between different mutants: fraction of piliated cells, average number of pili per piliated cell, and average pilus length.

Hemagglutination Titers. HA titers were performed as described in ref. 18, with minor modifications. Briefly, 1 mL of an OD_{600} 1.0 suspension of bacterial cells in PBS was gently pelleted (4,000 × *g*, 2 min) and resuspended in 100 μL PBS. Twenty-five microliters of this was serially diluted in a row of a 96-well V-bottom plate where each well contained 25 μL PBS (dilution range 1:2 to 1:4,096). Twenty-five microliters of guinea pig red blood cells were added to each well, agitated, and incubated overnight at 4 °C. The HA titer reported was the greatest dilution of cells that resulted in visible clumping of erythrocytes.

Mannose-Conjugated BSA ELISA. Cells were prepared as for HA titers. Ninety-six-well ELISA plates were coated at 37 °C for 1 h with 0.8 μg BSA-mannotriose, BSA-mannose, or bovine RNaseB in 100 μL PBS. Wells were washed three times with 200 μL 0.005% Tween-20 in PBS and blocked with 200 μL of 4% milk in PBS for 2 h at 37 °C. One hundred microliters of a 2-fold dilution series (dilution range 1:1 to 1:2,048) of cell suspension was added to each row of the ELISA plate, bound for 1 h at 37 °C while shaking at 100 rpm, then washed three times in 200 μL PBS. Detection was done by adding polyclonal rabbit anti-FimH sera (1:500 dilution) for 30 min at 37 °C, washing three times with 200 μL PBS, adding a monoclonal AP-conjugated goat anti-rabbit IgG antibody (1:1,000 dilution) for 2 h at 37 °C, and washing again with PBS. Wells were subsequently washed two times with 200 μL PBS and twice with ELISA buffer (100 mM Tris, pH 9.5; 50 mM $MgCl_2$; and 100 mM NaCl). One hundred microliters of 2 mg/mL *para*-nitrophenylphosphate (Sigma) in ELISA buffer was added for 30 min and absorbance at 405 nm was measured. The titer reported was the greatest dilution of cells that exceeded a baseline absorbance, calculated as three standard deviations above the mean fluorescence of control wells that contained no bacteria.

Binding and Invasion to Cultured Bladder Epithelial Cells. This assay was performed as described in refs. 19 and 20.

In Vivo Fitness Assay. Infection of 7- to 8-week-old C3H/HeN mice was performed as described in ref. 21, with minor modifications. Briefly, bacteria were grown under type 1 inducing conditions, harvested, then resuspended to an OD600 of 0.5 in PBS. Fifty microliters of this suspension was instilled transurethrally into the bladder. After 24 h, mice were killed, and bladders were aseptically removed and homogenized in 1 mL PBS. Serial dilutions were plated and total bacterial load per bladder was calculated.

In Vivo IBC Formation Assay. This assay was performed as described in ref. 22.

In Vivo Gentamicin Protection Assay. This assay was performed according to (21) with minor modifications. Briefly, cells were prepared and mice infected as described above for the in vivo fitness assay. One hour postinfection, mice were killed and bladders were aseptically removed. Bladders were hemisected and washed three times with 500 μL PBS. These three washes were combined and plated to obtain titers for loosely adherent and extracellular bacteria (Wash 1). Washed bladders were then incubated with shaking for 90 min at 37 °C in 100 μg/mL gentamicin. Bladders were then washed twice with 1 mL PBS to remove gentamicin, homogenized in 1 mL PBS, and titered. Bacterial titers from the bladder homogenate were presumed to represent intracellular bacteria.

In Vivo Competitive Gastrointestinal Colonization Assay. Bacteria were grown under type 1 inducing conditions. Two hundred microliters of an equal mixture of UTI89 (kanamycin sensitive) and a mutant strain (kanamycin resistant) carrying either a wild-type *fimH* or the A27V/V163A allele in PBS (2×10^8 total CFUs/200 μL) was gavaged into 6- to 8-week-old C57/Bl6 female mice (five mice per strain). Mice for each group were housed in separate gnotobiotic isolators. At 1, 4, 7, 11, and 14 days post-gavage, fecal pellets were collected from each mouse, homogenized, and titered on nonselective media for total CFUs. Fifty individual colonies were then assayed for kanamycin resistance to measure the ratio of mutant (kanamycin resistant) to UTI89 (kanamycin sensitive) titers.

Coinfection with GFP-Producing FimH Mutant Strains. FimH mutant strains were transformed with the pComGFP plasmid as described in ref. 23. Coinfection of mice, bladder harvest and staining, and confocal microscopy were performed as described in ref. 24.

Supporting Discussion. FimH mutations have been previously tested for mannose binding affinity in several studies (12, 25–28). Our mannose binding assays are largely consistent with these previous reports; discrepancies noted here may be due to one of several possibilities: (*i*) different *E. coli* strains; (*ii*) different background FimH amino acid sequence (not previously tested systematically); and (*iii*) differences in phase regulation.

We found that all FimH mutants have strong binding to tri-mannose (Fig. S3), as previously reported. Mutation of Ala-27 to Val-27 has been shown to cause a change from low- to high-mannose binding (12). Using FimH sequences identical to those previously reported, we do not consistently see the same effect (Fig. S3, mutants 7 and 15). However, by comparing mutants 5, 6, and 8 with mutants 13, 14, and 16, respectively, in Fig. S3, our results extend previous studies and demonstrate that there is, indeed, consistently lower mono-mannose binding affinity for FimH variants containing Ala-27 compared with those possessing Val-27. This result holds when position 27 is mutated in several different FimH sequences.

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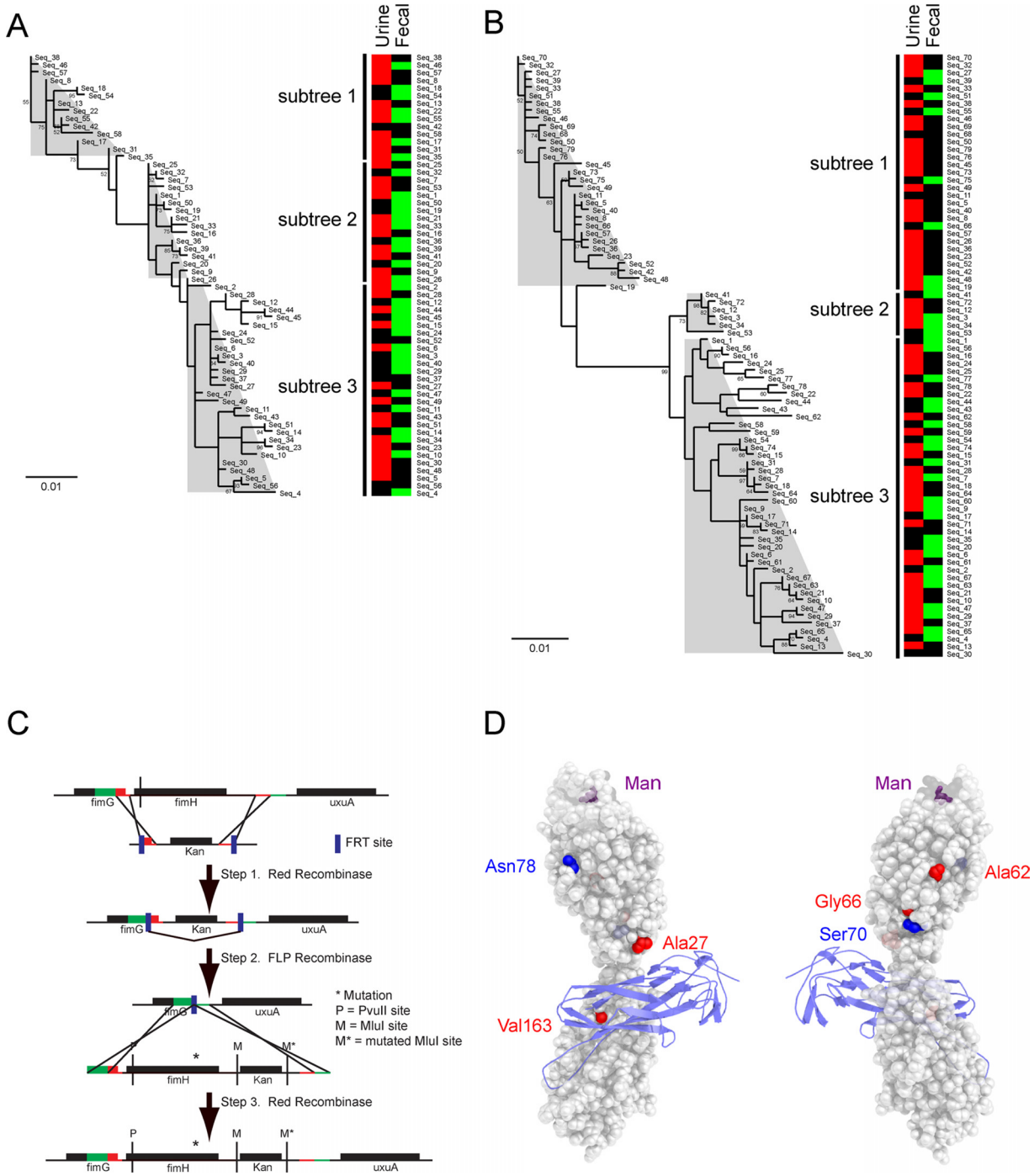

Fig. S1. Maximum likelihood trees, cloning scheme, and crystal structure. Maximum likelihood trees for *fimC* (*A*) and *fimH* (*B*). Unique sequences are labeled as arbitrarily assigned allele numbers (58 total for *fimC* and 78 for *fimH*, see Table S1). Bootstrap values (100 replicates) are indicated below and to the left of their respective nodes. Light gray trapezoids indicate manually identified subtrees. (Scale bar at lower left, number of mutations per nucleotide.) Color map indicates branches that include *fimC* or *fimH* sequences from urine (red) and fecal (green) strains, with black indicating nodes that were found in no urine or fecal strains (some *fimH* sequences are from strains not isolated from either urine or feces, see Table S1). (*C*) Scheme for construction of *fimH* mutants. The chromosomal region of UTI89 surrounding *fimH* is shown in each step. Before Steps 1 and 3, a linear piece of DNA is shown below the chromosomal map. Red and green areas represent homologous DNA sequences. Angled crossing black lines represent homologous recombination. Thicker blue vertical bars represent FRT (Flp recombinase target) sites. A mutation in *fimH* is represented by an asterisk. Kan is a kanamycin resistance cassette. P, *Pvu*II restriction site. M, *Mlu*I restriction site. M*, mutated MluI restriction site. (*D*) Location of positively selected and mutated amino acids. Space-filled representation of crystal structure of FimH in complex with FimC (ribbon representation) is shown (derived from PDB 1KLF). PSAA residues mutated (27, 62, 66, and 163) are colored and labeled in red. Non-PSAA residues mutated (70 and 78) are colored and labeled in blue. Bound mannose (purple, Man) is shown at the top, embedded within the mannose-binding pocket (dark gray). Image on the *Right* is rotated 180° about the *y* axis relative to the image on the *Left*.

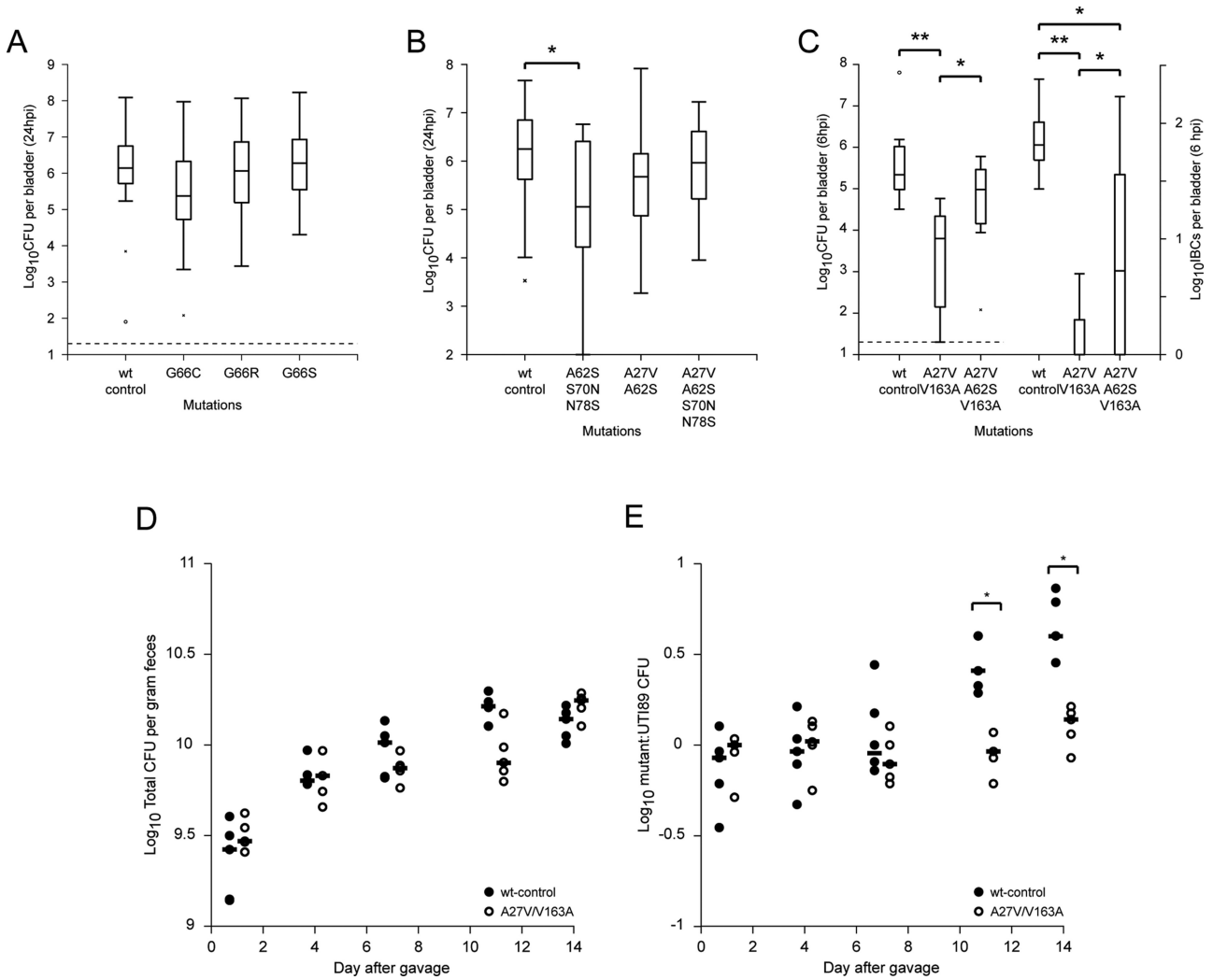

Fig. S2. In vivo fitness assays. (*A* and *B*) CFUs/bladder at 24 hpi. Mutations present are shown on the *x* axis. The *y* axis indicates the logarithm (base 10) of the bacterial CFUs measured in mouse bladders 24 hpi. Data are represented as box-and-whisker plots summarizing data from 15 to 20 mice for each strain. *, $P < 0.05$, **, $P < 0.001$, two-tailed Mann–Whitney test. (*C*) In vivo CFUs and IBC formation at 6 hpi. Mutations present are shown on the *x* axis. Left three lanes and *y* axis plot CFUs/bladder at 6 hpi. Dotted line represents the limit of detection. Right three lanes and *y* axis show IBCs formed per bladder. Data are represented as box-and-whisker plots summarizing data from 10 mice for each strain. *, $P < 0.05$, **, $P < 0.001$, two-tailed Mann–Whitney test. (*D* and *E*) Competition assays for gut colonization in gntobiotic mice. (*D*) Total bacterial load in feces. *y* axis plots the log (base 10) of the total colony forming units per gram of feces. Each dot represents one fecal pellet from one mouse. Filled circles represent data for mice colonized with UTI89 and the wt-control strain. Open circles represent data for mice colonized with UTI89 and the A27V/V163A mutant. Black bars indicate medians. (*E*) Relative abundance of mutant and UTI89 colonies recovered from feces. The number of days after gavage is shown on the *x* axis. *y* axis plots the log (base 10) of the ratio of mutant (antibiotic resistant) colonies to wild-type parental UTI89 (antibiotic sensitive) colonies in fecal pellets. Each dot represents one fecal pellet from one mouse. *, $P < 0.05$, two-tailed Mann–Whitney test.

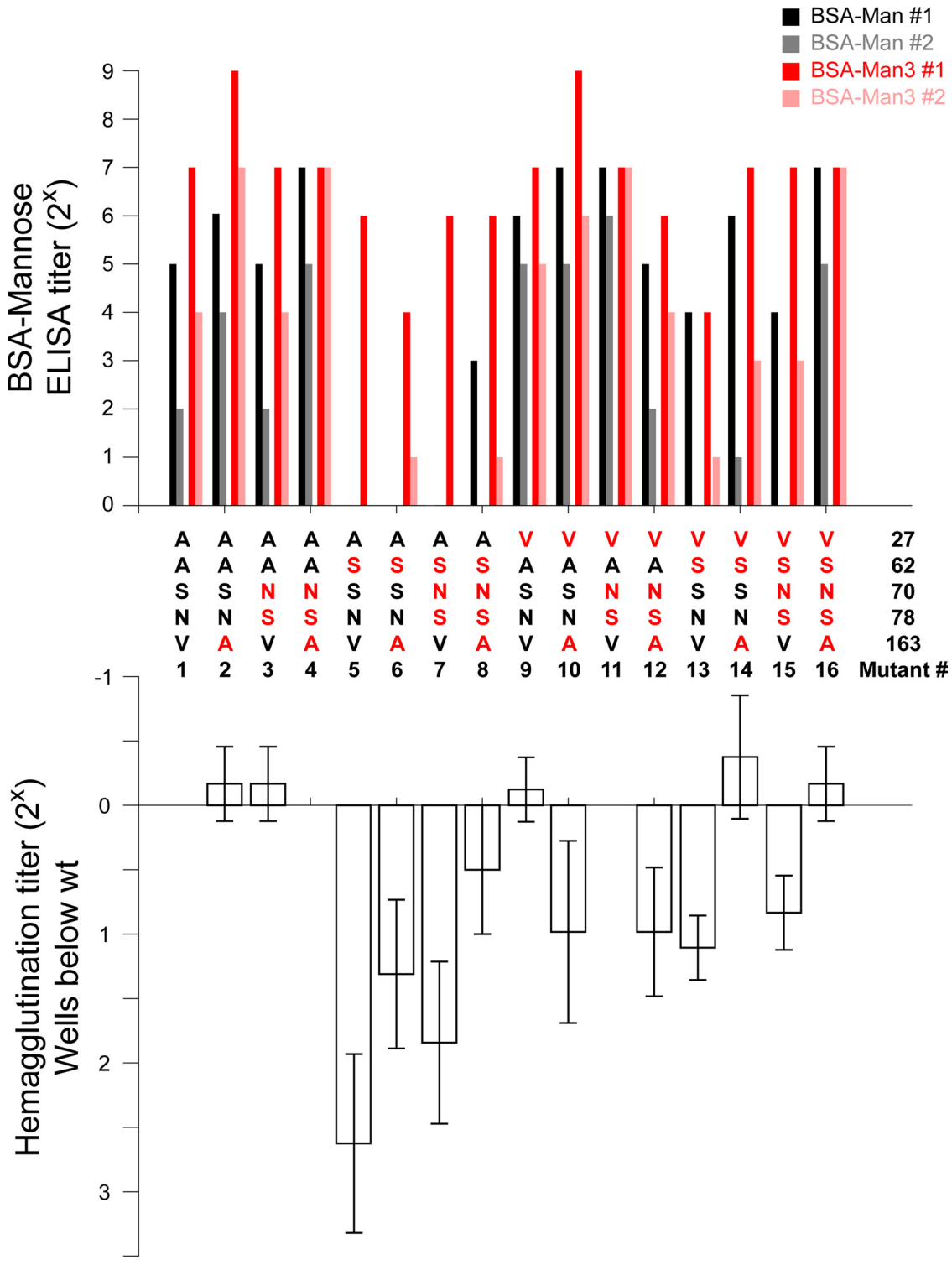

Fig. S3. In vitro mannose binding ELISA and hemagglutination assays. FimH sequence at positions 27, 62, 70, 78, and 163 are indicated on the *x* axis. Black indicates that the sequence is identical to the UTI89 FimH allele at that position, and red indicates a mutation. An arbitrary mutant number is also assigned for ease of reference. Upper panel indicates the log (base 2) of the titer for binding to BSA conjugated with mannose. Black/gray bars indicate binding titer in each of two independent experiments to BSA conjugated to monomannose. Red/pink bars, titer in two experiments for BSA conjugated to mannotriose. Lower panel indicates the log (base 2) of the decrease in hemagglutination (HA) titer relative to UTI89. Mean decrease is represented by bars, error bars indicate standard deviation. Each bar, at least $n = 3$ experiments.

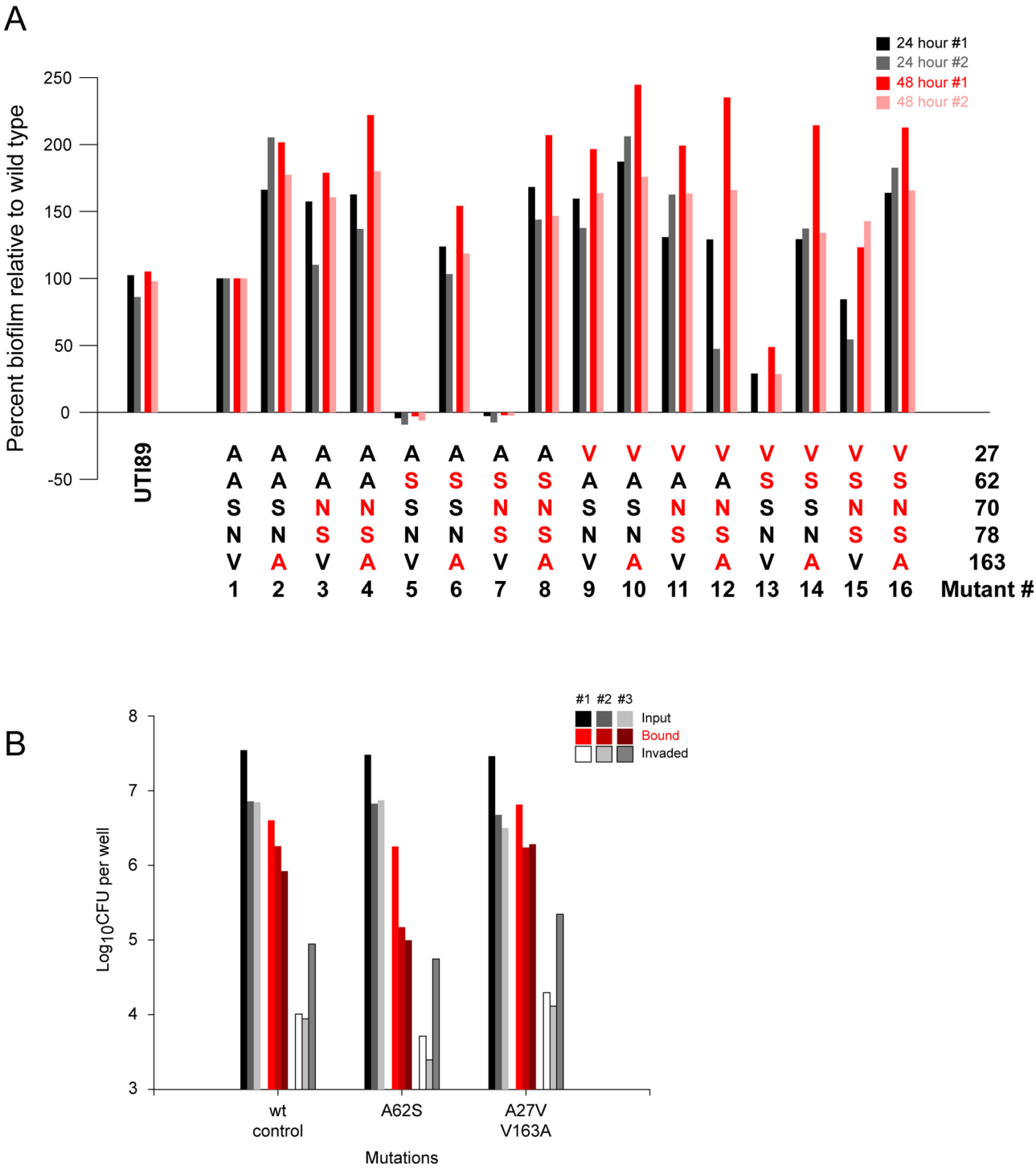

Fig. S4. In vitro biofilm formation and binding and invasion assays. (*A*) FimH sequence at positions 27, 62, 70, 78, and 163 and mutant number are indicated on the *x* axis as in Fig. S3. UTI89 indicates the parental UTI89 strain. The *y* axis plots the percentage of biofilm formation relative to a strain carrying the wild-type UTI89 *fimH* allele (mutant number 1) as measured by crystal violet staining. Black/gray bars represent mean biofilm formation of triplicate measurements after 24 h in two separate assays. Red/pink bars represent biofilm formation of triplicate measurements after 48 h in two separate assays. (*B*) In vitro binding and invasion assay. Tested strains are indicated on the *x* axis. The *y* axis plots the logarithm of bacterial CFUs per well. Black/gray bars represent bacterial CFUs added to cultured 5637 (human) bladder epithelial cells. Red/pink bars, CFUs remaining bound to 5637 cells after washing. White/gray bars outlined in black represent CFUs remaining after gentamicin treatment, indicating invasion. Bars, the average of three wells. Data from three independent experiments are shown.

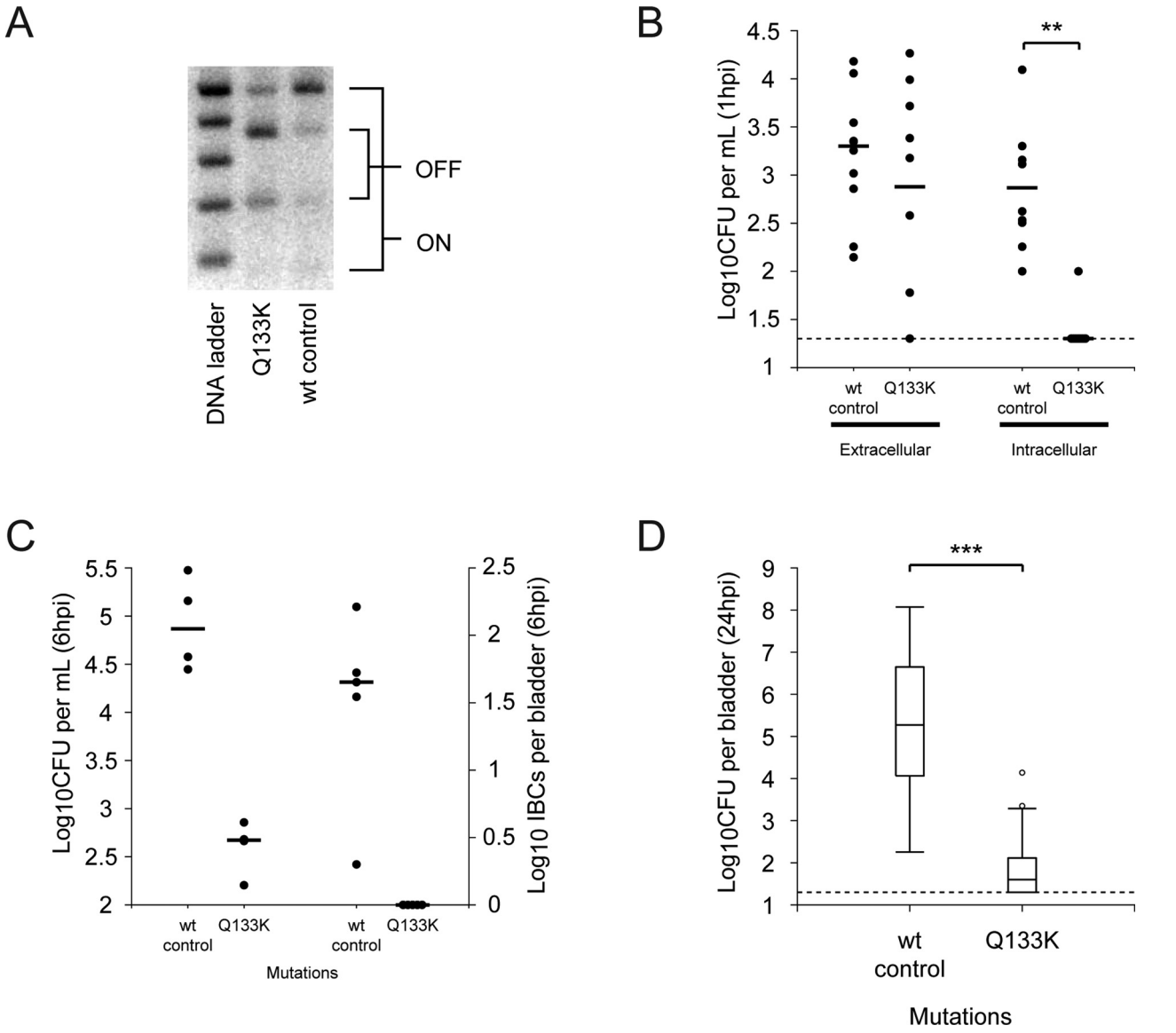

Fig. S5. Phase assay and in vivo fitness of Q133K (FimH mannose-binding pocket mutant) strain. (*A*) Phase assay under type 1 inducing conditions. Left lane contains DNA markers from 100 to 500 bp at 100-bp intervals. Phase ON and phase OFF bands are indicated on the right. FimH mutant strain is indicated at the bottom. (*B*) In vivo gentamicin protection assay. Mutations present in tested strains are shown on the *x* axis. The *y* axis plots the logarithm (base 10) of the number of bacterial CFUs per mL of wash or homogenate. The two left lanes show CFUs per mL of wash solution, representing luminal and loosely bound extracellular bacteria in the bladder. Right two lanes are CFUs per bladder after treatment with gentamicin and homogenization in 1 mL PBS, representing intracellular bacteria. (*C*) In vivo CFUs and IBC formation at 6 hpi. Mutants indicated on the *x* axis. Left two lanes and *y* axis are logarithm of CFUs/bladder at 6 hpi. Right two lanes and *y* axis display IBCs formed per bladder. Bladders with no IBCs detected are graphed as having one IBC. (*D*) Twenty-four hours postinfection in vivo fitness test. The FimH mutant strain is indicated on the *x* axis. *y* axis indicates the logarithm (base 10) of the bacterial CFUs measured in mouse bladders at 24 hpi. The dotted line represents the limit of detection. Data are represented as box-and-whisker plots summarizing results from 30 to 40 mice per strain. **, $P < 0.001$, ***, $P < 0.0001$, two-tailed Mann–Whitney test.

Table S1. List of strains sequenced

Genome sequences

fimH allele	*fimC* allele	Name	GenBank Accession	*fimH* subtree	*fimC* subtree
7	29	K12	NC_000913	3	3
7	29	W3110	AC_000091	3	3
14	5	EDL933	NC_002655	3	3
27	17	CFT073	NC_004431	1	1
30	52	Shigella flexneri 2a 2457T	NC_004741	3	3
30	52	Shigella flexneri 2a 301	NC_004337	3	3
68	8	APEC O1	NC_008563	1	1
69	8	UTI89	NC_007946	1	1

Laboratory strains

fimH allele	*fimC* allele	Original Name	Isolation Location	*fimH* subtree	*fimC* subtree
7	29	MG1655	Feces	3	3
69	8	UTI89	Urine	1	1
33	17	ASB1298	Urine	1	1
27	17	CFT073	Urine	1	1
14	5	EDL933		3	3
69	8	NU14	Urine	1	1
7	6	J96	Urine	3	3
27	17	DS17		1	1
69	8	EC45		1	1
65	54	E80		3	1
7	37	GR12		3	3
73	55	536	Urine	1	1

Clinical strains

fimH allele	*fimC* allele	Original Name	Isolation Location	Isolation Species	Study/ Collection	Patient Number	*fimH* subtree	*fimC* subtree
3	33	PY2	Urine	Human Female			2	2
60	26	PY3	Urine	Human Female			3	2
3	33	TOP115	Urine	Human Female	TOP	2	2	2
56	41	TOP320	Urine	Human Female	TOP	2	3	2
50	8	TOP409	Urine	Human Female	TOP	5	1	1
7	6	TOP207	Urine	Human Female	TOP	5	3	3
67	15	TOP599	Urine	Human Female	TOP	9	3	3
62	21	TOP227	Urine	Human Female	TOP	9	3	2
62	21	TOP578	Urine	Human Female	TOP	11	3	2
65	9	TOP263	Urine	Human Female	TOP	11	3	2
65	9	TOP799	Urine	Human Female	TOP	12	3	2
65	9	TOP876	Urine	Human Female	TOP	12	3	2
7	6	TOP379	Urine	Human Female	TOP	12	3	3
7	6	TOP291	Urine	Human Female	TOP	13	3	3
7	6	TOP498	Urine	Human Female	TOP	13	3	3
27	17	TOP344	Urine	Human Female	TOP	17	1	1
27	17	TOP746	Urine	Human Female	TOP	17	1	1
73	55	TOP345	Urine	Human Female	TOP	20	1	1
73	55	TOP493	Urine	Human Female	TOP	20	1	1
73	55	TOP587	Urine	Human Female	TOP	20	1	1
73	55	TOP356	Urine	Human Female	TOP	21	1	1
73	55	TOP673	Urine	Human Female	TOP	21	1	1
45	13	TOP596	Urine	Human Female	TOP	26	1	1
45	13	TOP1090	Urine	Human Female	TOP	26	1	1
9	15	TOP1002	Urine	Human Female	TOP	31	3	3
65	9	TOP1074	Urine	Human Female	TOP	31	3	2
27	17	UTI 3	Urine	Human Female			1	1
65	9	UTI 8	Urine	Human Female			3	2
34	16	UTI 10	Urine	Human Female			2	2
65	9	UTI 28	Urine	Human Female			3	2
63	15	UTI 4	Urine	Human Female			3	3
69	8	ASB 408	Urine	Human Female			1	1

Clinical strains

fimH allele	*fimC* allele	Original Name	Isolation Location	Isolation Species	Study/ Collection	Patient Number	*fimH* subtree	*fimC* subtree
42	57	ASB 452	Urine	Human Female			1	1
60	26	ASB 640	Urine	Human Female			3	2
73	55	ASB 1061	Urine	Human Female			1	1
32	17	ASB 1273	Urine	Human Female			1	1
16	41	ASB 1286	Urine	Human Female			3	2
33	17	ASB 1297	Urine	Human Female			1	1
69	8	ASB 131	Urine	Human Female			1	1
46	17	ASB 167	Urine	Human Female			1	1
65	9	ASB 229	Urine	Human Female			3	2
65	9	ASB 389	Urine	Human Female			3	2
60	26	ASB 556	Urine	Human Female			3	2
69	8	ASB 586	Urine	Human Female			1	1
73	55	ASB 957	Urine	Human Female			1	1
60	26	ASB 1134	Urine	Human Female			3	2
60	26	ASB 1158	Urine	Human Female			3	2
67	43	ASB 271	Urine	Human Female			3	3
7	6	ASB 277	Urine	Human Female			3	3
78	7	ASB 278	Urine	Human Female			3	2
79	55	ASB 506	Urine	Human Female			1	1
69	8	ASB 781	Urine	Human Female			1	1
57	31	ASB 1111	Urine	Human Female			1	1
73	55	ASB 1135	Urine	Human Female			1	1
32	17	ASB 1282	Urine	Human Female			1	1
60	26	ASB 933	Urine	Human Female			3	2
25	28	ASB 934	Urine	Human Female			3	3
18	6	ASB 941	Urine	Human Female			3	3
60	26	ASB 942	Urine	Human Female			3	2
18	6	ASB 950	Urine	Human Female			3	3
18	6	ASB 951	Urine	Human Female			3	3
32	17	ASB 1231	Urine	Human Female			1	1
61	51	ASB 451	Urine	Human Female			3	3
59	30	ASB 795	Urine	Human Female			3	3
76	55	ASB 967	Urine	Human Female			1	1
7	6	ASB 1230	Urine	Human Female			3	3
7	6	rASB 1154–1	Urine	Human Female			3	3
7	6	rASB 1154–2	Urine	Human Female			3	3
7	6	rASB 1154–3	Urine	Human Female			3	3
57	31	rASB 1021–2	Urine	Human Female			1	1
57	31	rASB 1021–2	Urine	Human Female			1	1
57	31	rASB 1021–3	Urine	Human Female			1	1
60	26	rASB 2289–1	Urine	Human Female			3	2
60	26	rASB 2289–2	Urine	Human Female			3	2
60	26	rASB 2289–3	Urine	Human Female			3	2
33	17	ASB 1298	Urine	Human Female			1	1
15	34	TB226A	Urine	Human Female			3	3
47	48	TB334C	Urine	Human Female			3	3
28	23	TBUTI01	Urine	Human Female			3	3
15	34	TB352C	Urine	Human Female			3	3
71	5	TB2755	Urine	Human Female			3	3
12	1	TB154A	Urine	Human Female			2	2
15	34	TB285A	Urine	Human Female			3	3
34	21	ECOR1	Feces	Human Female	ECOR		2	2
34	21	ECOR2	Feces	Human Male	ECOR		2	2
65	18	ECOR3	Feces	Dog	ECOR		3	1
43	21	ECOR5	Feces	Human Female	ECOR		3	2
9	44	ECOR7	Feces	Orangutan	ECOR		3	3
34	21	ECOR8	Feces	Human Female	ECOR		2	2
3	33	ECOR9	Feces	Human Female	ECOR		2	2
74	34	ECOR10	Feces	Human Female	ECOR		3	3
18	6	ECOR11	Urine	Human Female	ECOR		3	3
7	6	ECOR12	Feces	Human Female	ECOR		3	3
7	6	ECOR14	Urine	Human Female	ECOR		3	3

Clinical strains

fimH allele	*fimC* allele	Original Name	Isolation Location	Isolation Species	Study/ Collection	Patient Number	*fimH* subtree	*fimC* subtree
35	19	ECOR16	Feces	Leopard	ECOR		3	2
54	40	ECOR17	Feces	Pig	ECOR		3	3
4	35	ECOR18	Feces	Celebese ape	ECOR		3	1
34	21	ECOR19	Feces	Celebese ape	ECOR		2	2
54	3	ECOR20	Feces	Steer	ECOR		3	3
54	3	ECOR21	Feces	Steer	ECOR		3	3
35	50	ECOR23	Feces	Elephant	ECOR		3	2
7	6	ECOR24	Feces	Human Female	ECOR		3	3
74	34	ECOR25	Feces	Dog	ECOR		3	3
6	44	ECOR26	Feces	Human	ECOR		3	3
6	45	ECOR27	Feces	Giraffe	ECOR		3	3
6	44	ECOR28	Feces	Human Female	ECOR		3	3
6	14	ECOR29	Feces	Kangaroo rat	ECOR		3	3
34	21	ECOR30	Feces	Bison	ECOR		2	2
31	20	ECOR31	Feces	Leopard	ECOR		3	2
34	21	ECOR32	Feces	Giraffe	ECOR		2	2
34	21	ECOR33	Feces	Sheep	ECOR		2	2
6	10	ECOR34	Feces	Dog	ECOR		3	3
65	54	ECOR35	Feces	Human Male	ECOR		3	1
65	54	ECOR36	Feces	Human Female	ECOR		3	1
35	26	ECOR37	Feces	Marmoset	ECOR		3	2
29	2	ECOR39	Feces	Human Female	ECOR		3	3
29	2	ECOR40	Urine	Human Female	ECOR		3	3
17	47	ECOR42	Feces	Human Male	ECOR		3	3
41	1	ECOR43	Feces	Human Female	ECOR		2	2
1	36	ECOR44	Feces	Cougar	ECOR		3	2
67	15	ECOR45	Feces	Pig	ECOR		3	3
77	12	ECOR46	Feces	Celebese ape	ECOR		3	3
56	39	ECOR47	Feces	Sheep	ECOR		3	2
24	53	ECOR48	Urine	Human Female	ECOR		3	2
53	4	ECOR49	Feces	Human Female	ECOR		2	3
12	1	ECOR50	Urine	Human Female	ECOR		2	2
55	17	ECOR52	Feces	Orangutan	ECOR		1	1
7	6	ECOR53	Feces	Human Female	ECOR		3	3
39	17	ECOR54	Feces	Human	ECOR		1	1
39	17	ECOR55	Feces	Human Female	ECOR		1	1
60	26	ECOR56	Urine	Human Female	ECOR		3	2
27	17	ECOR57	Feces	Gorilla	ECOR		1	1
2	11	ECOR58	Feces	Lion	ECOR		3	3
66	55	ECOR59	Feces	Human Male	ECOR		1	1
5	55	ECOR60	Urine	Human Female	ECOR		1	1
7	6	ECOR61	Feces	Human Female	ECOR		3	3
7	6	ECOR62	Urine	Human Female	ECOR		3	3
19	22	ECOR63	Feces	Human Female	ECOR		1	1
7	6	ECOR64	Urine	Human Female	ECOR		3	3
75	55	ECOR65	Feces	Celebese ape	ECOR		1	1
48	46	ECOR66	Feces	Celebese ape	ECOR		1	1
20	24	ECOR67	Feces	Goat	ECOR		3	3
6	44	ECOR68	Feces	Giraffe	ECOR		3	3
58	26	ECOR69	Feces	Celebese ape	ECOR		3	2
7	6	ECOR70	Feces	Gorilla	ECOR		3	3
7	6	ECOR71	Urine	Human Female	ECOR		3	3
6	44	ECOR72	Urine	Human Female	ECOR		3	3
71	56	TB1828A	Urine				3	3
56	41	TOP326	Periurethra	Human Female	TOP	2	3	2
50	8	TOP413	Urine	Human Female	TOP	5	1	1
67	15	TOP690	Urine	Human Female	TOP	9	3	3
62	21	TOP699	Urine	Human Female	TOP	11	3	2
65	9	TOP855	Urine	Human Female	TOP	12	3	2
11	42	TOP845	Periurethra	Human Female	TOP	12	1	1
65	9	TOP870	Periurethra	Human Female	TOP	12	3	2
7	6	TOP294	Urine	Human Female	TOP	13	3	3

Clinical strains

fimH allele	*fimC* allele	Original Name	Isolation Location	Isolation Species	Study/ Collection	Patient Number	*fimH* subtree	*fimC* subtree
7	6	TOP295	Periurethra	Human Female	TOP	13	3	3
7	6	TOP542	Urine	Human Female	TOP	13	3	3
7	6	TOP537	Periurethra	Human Female	TOP	13	3	3
7	6	TOP920	Urine	Human Female	TOP	14	3	3
7	6	TOP924	Urine	Human Female	TOP	14	3	3
7	6	TOP925?	Periurethra	Human Female	TOP	14	3	3
27	17	TOP764	Urine	Human Female	TOP	17	1	1
27	17	TOP758	Periurethra	Human Female	TOP	17	1	1
73	55	TOP551	Periurethra	Human Female	TOP	20	1	1
73	55	TOP775	Urine	Human Female	TOP	21	1	1
73	55	TOP769	Periurethra	Human Female	TOP	21	1	1
45	13	TOP1126	Urine	Human Female	TOP	26	1	1
45	13	TOP1104	Periurethra	Human Female	TOP	26	1	1
9	15	TOP1069	Urine	Human Female	TOP	31	3	3
9	15	TOP1070	Periurethra	Human Female	TOP	31	3	3
69	8	TOP1187	Urine	Human Female	TOP	34	1	1
69	8	TOP1274	Urine	Human Female	TOP	34	1	1
69	8	TOP1250	Periurethra	Human Female	TOP	34	1	1
73	55	TOP1193	Urine	Human Female	TOP	35	1	1
73	55	TOP1207	Urine	Human Female	TOP	35	1	1
73	55	TOP1233	Urine	Human Female	TOP	35	1	1
73	55	TOP1218	Periurethra	Human Female	TOP	35	1	1
8	58	TOP1416	Urine	Human Female	TOP	41	1	1
8	58	TOP1490	Urine	Human Female	TOP	41	1	1
8	58	TOP1509	Urine	Human Female	TOP	41	1	1
8	58	TOP1501	Periurethra	Human Female	TOP	41	1	1
74	34	TOP1543	Urine	Human Female	TOP	41	3	3
74	34	TOP1561	Urine	Human Female	TOP	41	3	3
74	34	TOP1552	Periurethra	Human Female	TOP	41	3	3
65	9	TOP101	Urine	Human Female	TOP	1	3	2
27	17	TOP118	Urine	Human Female	TOP	3	1	1
22	25	TOP195	Urine	Human Female	TOP	6	3	2
65	9	TOP204	Urine	Human Female	TOP	8	3	2
34	16	TOP210	Urine	Human Female	TOP	10	2	2
73	55	TOP309	Urine	Human Female	TOP	15	1	1
52	38	TOP373	Urine	Human Female	TOP	22	1	1
36	31	TOP387	Urine	Human Female	TOP	23	1	1
7	6	TOP591	Urine	Human Female	TOP	25	3	3
67	15	TOP599	Urine	Human Female	TOP	9	3	3
57	31	TOP739	Urine	Human Female	TOP	27	1	1
65	9	TOP837	Urine	Human Female	TOP	28	3	2
26	31	TOP998	Urine	Human Female	TOP	30	1	1
65	9	TOP1006	Urine	Human Female	TOP	29	3	2
33	31	TOP1186	Urine	Human Female	TOP	36	1	1
56	39	TOP1290	Urine	Human Female	TOP	38	3	2
72	49	TOP1371	Urine	Human Female	TOP	39	2	3
47	10	TOP1378	Urine	Human Female	TOP	40	3	3
37	34	TOP1425	Urine	Human Female	TOP	42	3	3
65	9	TOP1564	Urine	Human Female	TOP	46	3	2
74	34	TOP1565	Urine	Human Female	TOP	45	3	3
74	34	TOP1580	Urine	Human Female	TOP	47	3	3
60	26	TOP1605	Urine	Human Female	TOP	48	3	2
27	17	TOP1609	Urine	Human Female	TOP	49	1	1
27	17	TOP1621	Urine	Human Female	TOP	49	1	1
60	26	TOP1648	Urine	Human Female	TOP	48	3	2
70	17	TOP1649	Urine	Human Female	TOP	50	1	1
63	44	TOP1650	Urine	Human Female	TOP	51	3	3
49	55	TOP1729	Urine	Human Female	TOP	53	1	1
74	34	TOP1829	Urine	Human Female	TOP	54	3	3
74	34	TOP1858	Urine	Human Female	TOP	54	3	3
10	30	TOP1950	Urine	Human Female	TOP	56	3	3
79	55	TOP1961	Urine	Human Female	TOP	57	1	1

Clinical strains

fimH allele	*fimC* allele	Original Name	Isolation Location	Isolation Species	Study/ Collection	Patient Number	*fimH* subtree	*fimC* subtree
74	34	TOP1987	Urine	Human Female	TOP	54	3	3
27	17	TOP2116	Urine	Human Female	TOP	56	1	1
73	55	TOP2161	Urine	Human Female	TOP	59	1	1
73	55	TOP2199	Urine	Human Female	TOP	59	1	1
48	46	TOP2205	Urine	Human Female	TOP	60	1	1
27	17	TOP2216	Urine	Human Female	TOP	56	1	1
6	30	TOP2293	Urine	Human Female	TOP	63	3	3
7	6	TOP2303	Urine	Human Female	TOP	65	3	3
7	6	TOP2315	Urine	Human Female	TOP	64	3	3
7	6	TOP2316	Urine	Human Female	TOP	64	3	3
69	8	TOP2339	Urine	Human Female	TOP	66	1	1
7	27	TOP2362	Urine	Human Female	TOP	67	3	3
63	44	TOP2363	Urine	Human Female	TOP	69	3	3
67	43	TOP2364	Urine	Human Female	TOP	70	3	3
52	38	TOP2385	Urine	Human Female	TOP	71	1	1
52	38	TOP2417	Urine	Human Female	TOP	71	1	1
60	26	TOP2433	Urine	Human Female	TOP	74	3	2
69	8	TOP2434	Urine	Human Female	TOP	73	1	1
73	55	TOP2445	Urine	Human Female	TOP	75	1	1
40	55	TOP2634	Urine	Human Female	TOP	78	1	1
38	17	TOP2652	Urine	Human Female	TOP	72	1	1
48	46	TOP2653	Urine	Human Female	TOP	79	1	1
65	9	TOP2801	Urine	Human Female	TOP	68	3	2
13	35	pyelo19	Urine	Human Female			3	1
21	30	pyelo31	Urine	Human Female			3	3
7	6	pyelo33	Urine	Human Female			3	3
57	31	pyelo37	Urine	Human Female			1	1
7	6	pyelo41	Urine	Human Female			3	3
19	22	1003 UTI	Urine	Human Female			1	1
47	44	1003 Rectal	Feces	Human Female			3	3
73	55	1182 UTI	Urine	Human Female			1	1
51	55	1182 Rectal	Feces	Human Female			1	1
23	13	1254 UTI	Urine	Human Female			1	1
44	32	1254 Rectal	Feces	Human Female			3	2
64	6	1266 UTI	Urine	Human Female			3	3
63	44	1266 Rectal1	Feces	Human Female			3	3
63	44	1266 Rectal2	Feces	Human Female			3	3
7	6	1333 UTI	Urine	Human Female			3	3
60	26	1333 Rectal	Feces	Human Female			3	2
65	35	17 Rectal1	Feces	Human Female			3	1
65	35	17 Rectal2	Feces	Human Female			3	1

Strain names, isolation locations (where known), and other information are shown. *fimH* and *fimC* alleles are coded as numbers that correspond to labels on phylogenetic trees in Fig. S1. The subtree in which the *fimH* and *fimC* sequences fall for each strain is indicated in the right two columns.

1. ECOR Ochman H, Selander RK (1984) Standard reference strains of Escherichia coli from natural populations. J Bacteriol 157:690–693
2. TOP Rosen DA, Hooton TM, Stamm WE, Humphrey PA, Hultgren SJ (2007) Detection of intracellular bacterial communities in human urinary tract infection. PLoS Med 4:e329.

Table S2. PAML results

Whole tree and branch-site models

Gene	Subtree	M1	M2	*p* value	M7	M8	*p* value	BSA	*p* value	w	amino acids (prob)	Bonferroni correction (# tests)
fimH		−2751.36	−2747.87	0.1526	−2751.71	−2747.77	0.0973					5
	1							−2748.42	0.2650			5
	1*							−2746.7	0.0473	10.7	27A (0.944), 62S (0.948), 74T (0.940), 163V (0.978)	5
	2							−2750.39	1.0000			5
	3							−2751.42	1.0000			5
fimC		−1773.09	−1772.49	1.0000	−1773.28	−1772.39	1.0000					4
	1							−1773.09	1.0000			4
	2							−1773	1.0000			4
	3							−1771.63	0.9292			4

Site models using subtrees

Gene	Subtree	M1	M2	*p* value	M7	M8	*p* value	w	proportion of codons under positive selection	amino acids (prob)	Bonferroni correction (# tests)
fimH	1	−1624.3	−1617.9	0.0067	−1624.5	−1617.9	0.0055	6.96	0.02983	27A (0.998), 62S (0.940), 66G (0.928), 74T (0.912), 163V (0.998)	4
	1*	−1555.48	−1546.97	0.0008	−1555.69	−1546.97	0.0007	9.12	0.02788	27A (0.999), 62S (0.958), 66G (0.951), 74T (0.940), 163V (0.999)	4
	2	−1293.37	−1293.37	1.0000	−1293.37	−1293.37	1.0000				4
	3	−2155.24	−2154.51	1.0000	−2155.45	−2154.52	1.0000				4
fimC	1	−1140.95	−1138.15	0.1823	−1141.09	−1138.15	0.1582				3
	2	−1146.11	−1145.62	1.0000	−1145.62	−1149.76	1.0000				3
	3	−1395.6	−1391.69	0.0600	−1395.69	−1391.95	0.0712				3

Top half shows data for site and branch-site models using the entire *fimH* or *fimC* tree. Bottom half shows data for site models applied to individual subtrees. FimH subtree 1* is subtree 1 but excluding the three sequences that do not encode for Ser70.

Table S3. Correlation of FimH amino acid variation with habitat from which the strain was isolated

Amino acid	Urine	Fecal	*p* value
70N, 78N	6	1	
70N, 78S	110†	53†	0.0001**
70S, 78N	72†	8†	
27A	149†	52†	0.3541
27T	0	1	
27V	39†	9†	
62A	13†	0†	0.0423*
62S	175†	62†	
66C	4	1	
66G	174†	57†	0.7533
66S	10†	4†	
163A	15†	1†	0.1287
163I	0	1	
163V	173†	60†	

The number of strains with *fimH* sequences predicted to code for each of the amino acid variants is shown. Cells marked by † were used in Fisher's exact test for correlation between amino acid variation and site of isolation, and the 2-tailed p-value is shown. * $P < 0.05$, ** $P < 0.001$.

Table S4. List of DNA primers, plasmids, and strains

DNA Primers

Name	Sequence	Notes
uti8+4908948	CTACCGGGCGTCGGGTTACT	Used to sequence fimC
uti8–4910078	CGATACGTCCCTGGCGGTAA	Used to sequence fimC
uti8–4910744	ATCACCGGGGCAAATCCTCT	Used to sequence fimC
uti8+4913338	ACCGCGCAAAACATCCAGTT	Used to clone fimH, sequence fimH, and for diagnostic PCR
uti8+4913927	CCGGTGGCGCTTTATTTGAC	Used to sequence fimH
uti8–4914820	GGTATTCGGCATTGGCCTGA	Used to clone fimH
uti8–4915222	GATCGTTTGGGCCGTACCAG	Used to sequence fimH and for diagnostic PCR
MluI-KanL	GATCACGCGTGGTTGGGAAGCCCTGCAAAG	Used to amplify and clone the kanamycin gene from pKD4, tailed with a MluI restriction site
AscI-KanR	GATCGGCGCGCCCGGTCATTTCGAACCCCAGA	Used to amplify and clone the kanamycin gene from pKD4, tailed with an AscI restriction site
uti8+4913515_fimH-pKD4-left	ATCACCTATACCTACAGCTGAACCCAAA-GAGATGATTGTAATGAAACGAGGTGTAG-GCTGGAGCTGCTTC	Used to knock out fimH
uti8–4914747_fimH-pKD4-right	CCTCTTAGATTACTTTGACCTGTCAGTA-AAAGACACGTTGAAAACCTGGGCATAT-GAATATCCTCCTTAG	Used to knock out fimH
uti8-phaseL	GAGAAGAAGCTTGATTTAACTAATTG	Used for type 1 phase assay
uti8-phaseR	AGAGCCGCTGTAGAACTCAGG	Used for type 1 phase assay
A27V-sew-L	TATGTAAACCTTGCGCCTGTGGTGAAT-GTGGGGCAAAAC	Used to make A27V mutation
A27V-sew-R	GTTTTGCCCCACATTCACCACAGGCGCAAGGTTTACATA	Used to make A27V mutation
A62S-sew-L	GTCACACTGCAACGAGGTAGCGCTTATG-GCGGCGTGTTA	Used to make A62S mutation
A62S-sew-R	TAACACGCCGCCATAAGCGCTACCTCGTTGCAGTGTGAC	Used to make A62S mutation
G66C-sew-L	CGAGGTGCGGCTTATGGCTGCGTGTTATCTAGTTTTTCC	Used to make G66C mutation
G66C-sew-R	GGAAAAACTAGATAACACGCAGCCATA-AGCCGCACCTCG	Used to make G66C mutation
G66R-sew-L	CGAGGTGCGGCTTATGGCCGTGTGTTATCTAGTTTTTCC	Used to make G66R mutation
G66R-sew-R	GGAAAAACTAGATAACACACGGCCATA-AGCCGCACCTCG	Used to make G66R mutation
G66S-sew-L	CGAGGTGCGGCTTATGGCAGCGTGTTATCTAGTTTTTCC	Used to make G66S mutation
G66S-sew-R	GGAAAAACTAGATAACACGCTGCCATA-AGCCGCACCTCG	Used to make G66S mutation
S70N/N78S-sew-L	GCTTATGGCGGCGTGTTATCTAACTTT-TCCGGGACCGTAAAATATAGCGGCAG-TAGCTATCCTTTCCCT	Used to make S70N/N78S mutation
S70N/N78S-sew-R	AGGGAAAGGATAGCTACTGCCGC-TATATTTTACGGTCCCGGAAAAGTTA-GATAACACGCCGCCATAAGC	Used to make S70N/N78S mutation
Q133K-sew-L	GCCGTGCTTATTTTGCGAAAAACCAACAACTATAACAGC	Used to make Q133K mutation
Q133K-sew-R	GCTGTTATAGTTGTTGGTTTTTCGCAAAATAAGCACGGC	Used to make Q133K mutation
V163A-sew-L	CCCACTGGCGGCTGCGATGCGTCTGCTCGTGATGTCACC	Used to make V163A mutation
V163A-sew-R	GGTGACATCACGAGCAGACGCATCG-CAGCCGCCAGTGGG	Used to make V163A mutation
FimBE KO #1	GGCAGGAATAATCGCTAGGGACCTAAG-CATTAGCATGATAATAGCGTGTAGGCTGGAGCTGCTTC	Used to knock out fimB and fimE, from Hannan et al. (14)
FimBE KO #2	GTCTTGTATTTATTTGTTTTTTAACTT-TATTATCAATTAGTTAAAATGGGAATTAGCCATGGTCC	Used to knock out fimB and fimE, from Hannan et al. (14)

Plasmids

Name	Parent plasmid	Notes	Source
pKM208		Source of Red recombinase	Murphy and Campellone (3)
pKD4		Source of kanamycin resistance cassette	Datsenko and Wanner (2)
pCP20		Source of FLP recombinase	Cherepanov and Wackernagel (13)
pUC19		General cloning vector	NEB
pCR4-TOPO		General cloning vector	Invitrogen
pSLC2–12-fimH-TOPO-1	pCR4-TOPO	Contains fimH sequence from UTI89	This study
pSLC2–16–2	pSLC2–12-fimH-TOPO	Contains fimH sequence and kanamycin resistance cassette	This study

Plasmids

Name	Parent plasmid	Notes	Source
pSLC2–24–3	pUC19, pSLC2–16–2	Contains fimH sequence and kanamycin resistance cassette, moved into pUC19	This study

Strains

Name	Parent strain	Notes	Source
TOP10		General cloning strain	Invitrogen
SLC2–12–1	UTI89	Kan resistant, fimH knockout	This study
SLC2–14–1	SLC2–12–1	Kan sensitive, fimH knockout	This study
SLC2–17-fimH	SLC2–14–1	Contains pKM208 plasmid for expression of Red recombinase system	This study
SLC2–33–1	SLC2–17-fimH	Wild type control	This study
SLC2–35–1	SLC2–17-fimH	Q133K	This study
SLC2–37–10	SLC2–17-fimH	S70N, N78S	This study
SLC2–39–1	SLC2–17-fimH	A27V	This study
SLC2–45–4	SLC2–17-fimH	V163A	This study
SLC2–46–1	SLC2–17-fimH	A62S	This study
SLC2–46–6	SLC2–17-fimH	A62S, S70N, N78S	This study
SLC2–51–1–1	SLC2–17-fimH	A27V, V163A	This study
SLC2–68–4–1	SLC2–17-fimH	A27V, A62S, S70N, N78S	This study
SLC2–68–6–1	SLC2–17-fimH	A27V, A62S	This study
SLC2–68–48–1	SLC2–17-fimH	A27V, S70N, N78S	This study
SLC2–68–184–1	SLC2–17-fimH	S70N, N78S, V163A	This study
SLC2–73–4–1	SLC2–17-fimH	A27V, A62S, S70N, N78S, V163A	This study
SLC2–73–48–1	SLC2–17-fimH	A27V, S70N, N78S, V163A	This study
SLC2–75–5–1	SLC2–17-fimH	A62S, S70N, N78S, V163A	This study
SLC2–76–2–1	SLC2–17-fimH	A62S, V163A	This study
SLC2–89–3	SLC2–17-fimH	A27V, A62S, V163A	This study
SLC4–16–1-C1	SLC2–17-fimH	G66C	This study
SLC4–16–1-R9	SLC2–17-fimH	G66R	This study
SLC4–16–1-S8	SLC2–17-fimH	G66S	This study

Primer name, sequence, and comments are shown. Plasmids and strains used in the construction of *fimH* mutants are listed. Parent strain or plasmid, notes on usage, and source are shown.