Allosteric Catch Bond Properties of the FimH Adhesin from Salmonella enterica Serovar Typhimurium*1

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Despite sharing the name and the ability to mediate mannose-sensitive adhesion, the type 1 fimbrial FimH adhesins of Salmonella Typhimurium and Escherichia coli share only 15% sequence identity. In the present study, we demonstrate that even with this limited identity in primary sequence, these two proteins share remarkable similarity of complex receptor binding and structural properties. In silico simulations suggest that, like E. coli FimH, Salmonella FimH has a two-domain tertiary structure topology, with a mannose-binding pocket located on the apex of a lectin domain. Structural analysis of mutations that enhance S. Typhimurium FimH binding to eukaryotic cells and mannose-BSA demonstrated that they are not located proximal to the predicted mannose-binding pocket but rather occur in the vicinity of the predicted interface between the lectin and pilin domains of the adhesin. This implies that the functional effect of such mutations is indirect and probably allosteric in nature. By analogy with E. coli FimH, we suggest that Salmonella FimH functions as an allosteric catch bond adhesin, where shear-induced separation of the lectin and pilin domains results in a shift from a low affinity to a high affinity binding conformation of the lectin domain. Indeed, we observed shear-enhanced binding of whole bacteria expressing S. Typhimurium type 1 fimbriae. In addition, we observed that anti-FimH antibodies activate rather than inhibit S. Typhimurium FimH mannose binding, consistent with the allosteric catch bond properties of this adhesin.

Terminal mannosyl residues are among the most common components of oligosaccharides in cell membrane glycoproteins of eukaryotic organisms, including humans. In particular, terminally exposed mannoside is ubiquitous in high mannose and hybrid types of N-linked oligosaccharides. Thus, it is not surprising that a great number of bacterial commensals and pathogens have evolved a variety of adhesive organelles that specifically recognize terminal mannoside, allowing them to attach to a wide spectrum of mammalian cells (1–5). For example, among different species of the Enterobacteriaceae, mannos-specific adhesion is mediated by type 1 fimbriae, relatively rigid fibrillar appendages of 0.5–2 μm in length with the mannose-binding protein (adhesin) positioned on the fimbrial tip. However, despite the general topological similarities, type 1 fimbriae in some bacterial species (e.g. Escherichia coli, Salmonella enterica, and Serratia marcescens) are not closely related genetically (6–9) and probably have evolved independently from each other as a result of convergent evolution driven toward the ability to bind terminal mannoside, highlighting the physiological importance of mannos-specific bacterial adhesion.

The mannos-specific adhesive properties of S. enterica type 1 fimbriae, similar to other enterobacterial species, depend on the tip-associated adhesin FimH, whereas the fimbrial shaft is primarily composed of the major structural subunit, FimA, that is not adhesive (10, 11). Comparison of fim gene clusters from S. Typhimurium and E. coli revealed that they differ substantially in chromosomal location, gene arrangement, and content (6, 12). Moreover, their nucleotide sequences virtually lack homology and show significant divergence in GC content and codon usage, indicating their independent evolutionary origins (6, 7).

In S. Typhimurium, the interactions between type 1 fimbriae and host cell receptors have been shown to promote bacterial adhesion in the process of host colonization and invasion (13–17). However, recent studies have demonstrated that naturally occurring minor variations in the FimH adhesins of S. Typhimurium type 1 fimbriae can result in drastic differences in bacterial binding to mammalian cells. It has been reported that type 1 fimbriae-mediated binding of S. Typhimurium strain LB5010 to HEp-2 cells was significantly higher than that of the S. Typhimurium strain SL1344 (14). Differences in binding phenotypes of these strains were recapitulated by each FimH allele expressed in an otherwise isogenic background, demonstrating that the phenotypes were not determined by possible differences in fimbrial shaft proteins or other fimbriae. FimHSL1344 differs from FimHLB5010 by only two amino substitutions, G39A and F96S, with the former being critical for the adhesive difference. Differential binding to HEp-2 cells also correlated directly with the ability of S. Typhimurium to form biofilms, both in vitro and in vivo. Bacteria expressing FimHSL1344 grew in a thick layer when attached to the Hep-2 cells monolayers and formed a distinct biofilm over intestinal epithelium in orally inoculated mice. Thus, the differential ability of S. Typhimurium FimH variants to mediate cell binding is likely to have significant physiological consequences. Another recent study reaffirmed that naturally occurring point mutations in FimH can have a

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profund effect on the bacteria (18). When the low binding S. Typhimurium strain SL1344 was compared with another type 1 fimbriated S. Typhimurium isolate, strain AJB3, only the latter was capable of binding efficiently to murine dendritic cells. The difference was again shown to be a function of the FimH allele of each strain. In that case, the FimH^{AJB3} variant differed from the FimH^{SL1344} by a single and different amino acid substitution, N136Y. It has also been shown that FimH^{AJB3} is essential for cellular uptake of bacteria by dendritic cells, survival in which is important for Salmonella pathogenesis (19). Thus, higher binding capability of FimH could lead to increased pathogenicity of S. enterica serovar Enteritidis fused with a C-terminal His tag) was kindly provided by Maciej Ugor- skii (Wroclaw, Poland). Anti-FimH Fab fragments were obtained from anti-FimH IgG (150 µg) by papain digestion using Pierce Fab Micro preparation kit according to the manufacturer’s instructions (Thermo Scientific). The rabbit Salmonella O group B antiserum (catalog no. 240984) was purchased from BD Bioscience.

Bacterial Strains and Plasmids—Recombinant strains of S. Typhimurium SL1344H3 and LBH4 (14, 27) were grown statically in SB medium at 37 °C overnight and supplemented with appropriate antibiotic when necessary. E. coli DH5α, used for all cloning experiments, was grown with shaking in SB medium supplemented with 30 µg/ml chloramphenicol.

Plasmid pISF255 carrying the fimH segment of S. Typhimurium SL1344 (14) was modified by insertion of an SpeI restriction site between the fimH and fimF genes. This was performed by site-directed mutagenesis of full-length pISF255 plasmid using the following pair of primers: fim-speF 5′-CGGCTATCTAATCTGATTGTAGATTTA-3′ and fim-speR 5′-TTAATCATATCGACTAGTATAAGCCGCTAGC-3′. The resulting plasmid, pISF255b, was used for XbaI/SpeI cloning of all fimH alleles analyzed in this study and construction of the fimH deletion mutant (fimHΔ). The fimH alleles of S. Typhimurium AJB3, S. Typhimurium LB5010, and single mutants fimH^{SL1344-G39A} and fimH^{SL1344-F96S} were obtained by site-directed mutagenesis of the fimH^{SL1344} gene using full-length fimH^{SL1344} plasmid and the following pairs of primers: N136Y-F (5′-GGCCATGGTTATATATGGTCAG-GAT-3′) and N136Y-R (5′-ATCGTACCATATAATAAAGGCC-3′); G39A-F (5′-GAAAAATCAGCTTGGTGCCTGCGGTAAACG-3′) and G39A-R (5′-GTTTACCCGACGACCAAGCTGATTTTTC-3′); and F96S-F (5′-TGCCTGTCATGCAGTATCTATCCGCGGC-3′) and F96S-R (5′-CCGGCCGCGATTAGATCGCCACGACGAC-3′). Standard recombinant DNA methods (28) or procedures specified by the manufacturer were used. The resulting plasmids were verified by DNA sequencing (University of Washington Sequencing Facility) using the BigDye Terminator version 3.1 cycle sequencing kit and appropriate vector-specific primers.

Random Mutagenesis of fimH—Random mutations were introduced into the fimH gene by error-prone PCR using GeneMorph II random mutagenesis kit (Stratagene, La Jolla, CA) and the following primers: fimH-XbaI-F (5′-CTCTTCTAGATGATCCGCCGTGC-3′) and fimH-SpeI-R (5′-GAGACTAGTTATACCATATGACTGC-3′). Plasmid pISF255b, carrying the fimH^{SL1344} allele, was used as a template. The PCR products were cloned into pISF255b vector as described above. The pool of constructs was first transformed into E. coli DH5α, and then plasmids isolated from chloramphenicol-resistant transformants were introduced into S. Typhimurium LTH4. The S. Typhimurium fimH mutant library was screened for RNase B^3 and yeast mannan binding in Sigma unless stated otherwise. The rabbit polyclonal anti-FimH antibody (protein A-purified IgG; raised against recombinant full-length FimH of S. enterica serovar Enteritidis fused with a C-terminal His tag) was kindly provided by Maciej Ugor-ski (Wroclaw, Poland). Anti-FimH Fab fragments were obtained from anti-FimH IgG (150 µg) by papain digestion using Pierce Fab Micro preparation kit according to the manufacturer’s instructions (Thermo Scientific). The rabbit Salmonella O group B antiserum (catalog no. 240984) was purchased from BD Bioscience.

**EXPERIMENTAL PROCEDURES**

Reagents and Antibodies—Mannose-BSA was obtained from Dextra Laboratories. All other reagents were obtained from

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3 The abbreviations used are: RNase B, ribonuclease B; α-mm, methyl-α-1-mannopyranoside.
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a static adhesion assay. The fimH clones with high and low binding phenotypes were selected and sequenced. Finally, the binding phenotype of all fimH clones was retested in a static adhesion assay using 3H-labeled bacteria.

Static Adhesion Assay—For screening of S. Typhimurium LTH4 fimH mutant library, bacterial colonies were picked up from agar plates and used for inoculation of 200 μl of LB medium. Bacteria were grown statically overnight at 37 °C and then spun and resuspended in 200 μl of phosphate-buffered saline (PBS). Bacterial suspensions at 50-μl volumes were added to each well of plates precoated with 20 μg/ml RNase B, yeast mannan, or 1:500 diluted anti-FimH antibody and incubated for 1 h at 37 °C. After washing, plates were dried in an air flow (~65 °C) for 10 min and visually scored.

FimH-dependent static adhesion of 3H-labeled bacteria was analyzed as described previously (24). Briefly, immulon 4HBX 12-well strips (Thermo Electron Corp.) were coated with 20 μg/ml mannose-BSA, RNase B, or rabbit anti-FimH antibody (diluted 1:500) and blocked with 0.1% BSA in PBS. Bacteria, seeded with the necessary antibiotics, were grown overnight with 0.33 μM [methyl-3H]thymidine (PerkinElmer Life Sciences). After washing, bacteria were resuspended in PBS and added to wells at a 100-μl volume and A560 = 2. Plates were washed with PBS, and radioactivity for each well was counted with a scintillation counter (MP Biomedicals). The number of bound bacteria was determined from calibration curves using BIAevaluation software (GE Healthcare). For inhibition, bacterial binding was tested in the presence of 50 mM methyl-α-D-mannopyranoside (α-mm).

Hep-2 Adhesion Assay—S. Typhimurium SL1344H3 expressing different FimH variants were added to Hep-2 cell monolayers at a multiplicity of infection of 50:1 in the presence or absence of 50 mM α-mm. Bacteria were allowed to interact with the cells for 1 h at 37 °C in 5% CO2. The cells were then washed five times with DMEM and lysed with 1% Triton (Sigma) in DMEM. The number of colony-forming units for each well was quantified by plating serial dilutions of cell lysates on LB plates. In some experiments, bacteria were first pre-treated with anti-FimH antibody (1:1000 dilution), anti-FimH Fab (1:1000 dilution), preimmune rabbit serum (1:100 dilution), or anti-Salmonella antigen O group B serum (1:100 or 1:20 dilutions) for 30 min at room temperature and then after vigorous pipetting added to Hep-2 monolayers.

The reactivity of anti-Salmonella antigen O group B serum with Salmonella cells in comparison with anti-FimH antibody was tested by ELISA (supplemental Fig. S1).

Parallel Plate Flow Chamber Experiment—Binding under different flow conditions was performed using a parallel plate flow chamber as described previously with minor modifications (29). Briefly, 35-mm polystyrene cell culture dishes (Corning Glass) were coated with mannose-BSA (200 μg/ml) or RNase B (100 μg/ml). A parallel plate flow chamber (2.5 cm (long) × 0.25 cm (wide) × 250 μm (high); GlycoTech) was assembled on top of the cell culture dish according to the manufacturer’s instructions. The entire assembly was then mounted on a Nikon TE2000-E microscope with a 10× phase-contrast objective, which was connected to a high resolution CCD Cascade camera (Roper Scientific, Inc.). Bacteria in 0.2% BSA-PBS were pumped into the chamber at different flow rates using a Warner Instruments syringe pump. Bacterial binding to the surface was recorded for 4 min at each respective flow rate and analyzed using MetaView video acquisition software (Universal Imaging Corp.).

Fimbrial Purification—Type 1 fimbriae were purified from S. Typhimurium LTH4 expressing different variants of FimH. Bacteria were grown overnight at 37 °C with shaking (100 rpm) in Terrific broth (Difco) supplemented with 30 μg/ml chloramphenicol. Bacterial cultures were harvested at 7000 rpm, resuspended in 50 mM Tris-HCl and 150 mM NaCl buffer at pH 7, and osterized four times for 1 min with a 1-min pause on ice using PRO 200 homogenizer (PRO Scientific Inc., Oxford, CT). Cell debris was then spun down, and fimbriae were precipitated from the supernatant with 0.2 M MgCl2 at least twice. Finally, fimbriae were dissolved in PBS. Protein concentration was measured using the BCA™ protein assay kit (Pierce) after the fimbriae were heated for 5 min at 99 °C in the presence of 0.1 M HCl.

Horseradish Peroxidase (HRP)-binding Assay—96-well microtiter plates were coated overnight with purified type 1 fimbriae in bicarbonate buffer at pH 9.6 (0.4 mg/ml) and 4 °C. The immobilized fimbriae were co-incubated with HRP (100 μg/ml), a mixture of HRP and 1:2000 diluted anti-FimH antibody, or a mixture of HRP and preimmune rabbit serum for 1 h at 37 °C. After extensive washing, the colorimetric reaction was developed by adding 3,3′,5,5′-tetramethylbenzidine (KPL) to the wells, and A was read at 650 nm. For inhibition, HRP binding was tested in the presence of 50 mM α-mm.

Homology Modeling—The amino acid sequence of S. Typhimurium FimHSL1344 (accession number CBW16639) was submitted to different fold recognition and template modeling servers: Phyre (30), I-Tasser (31), and Swiss-Model (32). The best ranked templates (Protein Data Bank codes 1KLF and 3JWN) and the template-query sequence alignment generated by Phyre were further used to construct a tertiary structure of Salmonella FimH by the Modeler program (33). The predicted model was viewed and analyzed using the molecular visualization system PyMOL. A cut-off of 4 Å was used for the interdomain contact residue determination in the low affinity conformation FimH model. For the analysis of distribution of mutations in the lectin domain, distances (in Å) were measured between the Ca atoms of each mutated amino acid and the nearest amino acid in the predicted binding pocket or interdomain contact.

Statistical Analyses—The Mann-Whitney U test (GraphPad Prism5) was used to determine the statistical significance of differences in distributions between the neutral and the binding-increasing mutations relative to distances from the interdomain interface of FimH. A p value of 0.05 (two-tailed) was considered to be statistically significant. For data from HRP and Hep-2 cell binding experiments, a parametric test (Student’s t test) was used to determine whether differences between treatment groups were statistically significant (p < 0.05).

RESULTS

Diverse FimH-dependent Adhesion of S. Typhimurium to Hep-2 Cells and Purified Mannose-containing Ligands—We constructed isogenic strains expressing three S. Typhimurium FimH variants that, as previously reported (14, 18), mediated
differential binding to mammalian cells. The bacteria expressed FimH from strain SL1344 (FimH<sup>SL1344</sup>) and its isogenic single (N136Y) and double (G39A/F96S) mutants representing wild type FimH from AJB3 and LB5010 strain, respectively. In accordance with previous results, the FimH<sup>SL1344</sup> variant mediated significantly lower bacterial cell adhesion to Hep-2 cells than FimH<sup>AJB3</sup> and FimH<sup>LB5010</sup> (Fig. 1A). Bacteria expressing different variants of FimH bound at the same level to polyclonal anti-FimH antibody, which indicated the same amino acid position as a natural variant targeting positions 136, 137, and 138 (Fig. 2). The enhancing mutations were distributed along the entire amino acid position. One mutation, F21S, occurred independently twice, in different mutant backgrounds. Overall, the binding-enhancing mutations were distributed along the entire length of the protein and did not exhibit a distinctly narrow clustering in the primary structure, except for three mutations targeting positions 136, 137, and 138 (Fig. 2). The enhancing mutations affected various types of amino acid residues and were of both a radical and conservative nature without significant correlation between the level of structural conservation of mutation and the level of binding enhancement. Thus, a rela-

![Figure 1](image_url)

**Figure 1. Static adhesion of S. Typhimurium expressing different variants of FimH.** Binding of bacteria to a monolayer of Hep-2 cells (A), mannosylated BSA (B), and RNase B (C) in the absence or presence of 50 mM α-mm. A non-fimbriated fimH mutant of S. Typhimurium strain SL1344H3 was complemented with pISF255b plasmids carrying different alleles of S. Typhimurium fimH or pISF255b plasmid with the fimH gene deletion (fimH<sup>D</sup>). Bacterial binding to Hep-2 cells, mannosylated BSA, and RNase B was determined as described under "Experimental Procedures." Data are the means ± S.E. (error bars) of triplicates from one representative experiment of three experiments that were performed.

The magnitude of difference between the strains in adhesion to Hep-2 cells more closely resembled the difference in mannosylated BSA binding (5–7-fold) than the difference in binding to RNase B (2-fold).

FimH<sup>LB5010</sup> differs from FimH<sup>SL1344</sup> in two amino acids (G39A and F96S); thus, we engineered single mutants, FimH<sup>SL1344-G39A</sup> and FimH<sup>SL1344-F96S</sup>, and investigated their binding properties. The substitution of glycine with alanine at position 39 of FimH<sup>SL1344</sup> resulted in a significant increase of bacterial adhesion, and the binding mediated by FimH<sup>SL1344-G39A</sup> mutant was even higher than that of the original FimH-G39A/F96S double mutant, especially to the mannosylated BSA substrate. In contrast, the substitution of phenylalanine with serine at position 96 caused a slight decrease in bacterial binding to both substrates relative to FimH<sup>SL1344</sup> (Fig. 1, A and B).

These results showed that the low and high adhering profiles of S. Typhimurium FimH variants to mammalian cells correspond well to the binding of model mannosylated substrates, in particular to mannosylated BSA with single terminal mannoside residues.

**Effect of Random Point Mutations on Binding Properties of S. Typhimurium FimH**—The availability of model receptor substrates allowed us to screen for additional structural changes that are capable of converting the low binding phenotype of FimH<sup>SL1344</sup> to high binding. We subjected FimH<sup>SL1344</sup> to random mutagenesis and investigated the binding phenotype of the resulting mutants to mannosylated BSA and RNase B. Of 200 clones screened in the functional assay, 20 clones with increased monomannose binding and 20 clones with unchanged monomannose binding were analyzed by sequencing the corresponding fimH gene copy. Because some of the mutagenized fimH copies contained multiple mutations (2–5 mutations), the changes responsible for the binding phenotype were defined by separating the mutations by site-directed mutagenesis and functional retesting.

Of 38 single mutants analyzed in the study, 15 exhibited 2–7-fold increases in binding to mannosylated BSA, accompanied by smaller increases in binding to RNase B (Table 1). Differences in mannoside-specific binding were not correlated with relatively minor variability in reactivity with anti-FimH antibodies.

We have identified a total of 15 new point mutations that enhance the binding phenotype of FimH<sup>SL1344</sup> variant and 23 mutations that do not significantly affect binding (neutral changes). Only one binding-enhancing mutation, N136D, targeted the same amino acid position as a natural variant (FimH<sup>AJB3</sup> (N136Y)) but was of a different nature. Similarly, two different mutations, R232L and R232W, targeted the same amino acid position. One mutation, F21S, occurred independently twice, in different mutant backgrounds. Overall, the binding-enhancing mutations were distributed along the entire length of the protein and did not exhibit a distinctly narrow clustering in the primary structure, except for three mutations targeting positions 136, 137, and 138 (Fig. 2). The enhancing mutations affected various types of amino acid residues and were of both a radical and conservative nature without significant correlation between the level of structural conservation of mutation and the level of binding enhancement. Thus, a rela-
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tively large number of additional mutations could be identified that significantly enhance FimHSL1344 binding but without a clear distribution pattern along the primary structure.

Modeling of Salmonella FimH Structure—To investigate a possible structural mechanism for the functional effects of mutations that convert the low binding FimH variants into high binding phenotypes, we generated a tertiary structural model of S. Typhimurium FimH. First, the amino acid sequence of FimHSL1344 was submitted to fold recognition and template modeling servers (Phyre, I-Tasser, Swiss-Model) that compare the query sequence and its predicted secondary structure with sequences and folds of proteins in the Protein Data Bank (30–32). Examination of the query-template sequence alignments generated by these programs showed that two alternative structures of E. coli FimH represent the adhesin in complex with the molecular chaperone FimC (1KLF) or as part of a multimeric fimbrial tip complex (3JWN). In both structures, the secondary and overall tertiary structures (two domains with β-sandwich fold) are very similar, but in the FimH-FimC complex, the lectin domain is wedged out from the pilin domain by the chaperone protein, whereas in the tip-associated FimH, the domains closely interact with one another. As a result of interdomain interaction, the lectin domain in the latter structure is in a more twisted and compressed conformation.

By using MODELLER software (33) and both E. coli FimH structures as alternative templates, a three-dimensional model of S. Typhimurium FimH was created that revealed an architecture highly similar to that of E. coli FimH, with two domains, an N-terminal domain including amino acids 1–173 (analogous to the mannose-binding lectin domain of the E. coli FimH) and a C-terminal domain, including amino acid residues 177–313 (analogous to the pilin domain of E. coli FimH), that are linked by a short, 3-amino acid peptide (Fig. 4, A and B). The cysteine residues form stabilizing disulfide bonds in the lectin (Cys34_Cys47 and pilin (Cys177_Cys220) domain as they do in the corresponding domains of E. coli FimH. On one hand, resemblance between the proteins of the general structural architectures is not surprising because the Salmonella FimH secondary structure is rich in β-strands, similar to E. coli FimH. On the other hand, the juxtaposition of four cysteines into two pairs for bridge formation at similar locations could be expected only if there is a high level of three-dimensional structural homology between the two proteins (the automatic version of Modeler used here does not explicitly restrain the location of cysteine). Importantly, threonine at position 56 that has been previously reported to be critical for mannose binding of S. enterica FimH (35) is located on one of the top loops of the N-terminal domain of the FimH model structure. According to the crystal structure of E. coli FimH and the pocket/clamp analysis generated by Phyre, this loop indeed corresponds to one of the top loops that form the man-

TABLE 1
Static adhesion of S. Typhimurium expressing different mutants of FimH

| FimH       | Mannose-BSA | RNase B | Anti-FimH antibody |
|------------|-------------|---------|--------------------|
|            | bacteria/well $\times 10^8$ |         |                    |
| T221A      | 7.2 ± 0.6   | 57.5 ± 2.1 | 125.2 ± 3.4        |
| V292M      | 7.3 ± 1     | 71.8 ± 2.6 | 114.5 ± 3.1        |
| F189I      | 8.7 ± 0.1   | 75.6 ± 2.6 | 162.2 ± 4.3        |
| K115M      | 9.3 ± 0.1   | 66.7 ± 1.1 | 135.8 ± 8.2        |
| D276A      | 10.5 ± 0.3  | 85.3 ± 1.7 | 123.5 ± 8.1        |
| A157T      | 10.9 ± 0.5  | 112.1 ± 3.2 | 155.6 ± 12.6       |
| I165F      | 11.5 ± 1.5  | 115.9 ± 21.5 | 164.9 ± 9        |
| V292E      | 11.2 ± 1.4  | 63.4 ± 4.5 | 180.3 ± 9.8        |
| Q226H      | 14.2 ± 0.2  | 87.6 ± 4.4 | 150.9 ± 0.7        |
| G259S      | 14.8 ± 1.3  | 69.1 ± 6.2 | 139.0 ± 12.1       |
| E178A      | 15.0 ± 1.7  | 75 ± 4   | 162.4 ± 4.2        |
| K129E      | 15.5 ± 0.5  | 55.7 ± 3.3 | 137.4 ± 2.6        |
| S122T      | 18.6 ± 0.7  | 62.7 ± 5.3 | 167.8 ± 7.8        |
| A281T      | 19.4 ± 1.3  | 68.3 ± 2.3 | 192.2 ± 7.5        |
| E186D      | 21.6 ± 1.1  | 117.8 ± 9.9 | 190.1 ± 1.1       |
| S257I      | 24.1 ± 1.1  | 104.9 ± 10.3 | 170.2 ± 2.7   |
| N258D      | 24.1 ± 0.3  | 112 ± 7.8 | 200.7 ± 2.6        |
| V185M      | 24.7 ± 0.7  | 79.9 ± 8.4 | 133.6 ± 17.7       |
| SL1344     | 26.4 ± 0.8  | 94.6 ± 5.7 | 145.4 ± 7.5        |
| Q212H      | 27.9 ± 3.7  | 107.2 ± 2.3 | 155.2 ± 6.2        |
| P301L      | 30.2 ± 1.1  | 119.7 ± 1.5 | 136.1 ± 1.1       |
| N5K        | 33.2 ± 2.2  | 92.2 ± 7   | 182.1 ± 3.6        |
| A93T       | 34.8 ± 4.8  | 147.9 ± 22.8 | 121.3 ± 2.6     |
| A10V       | 34.3 ± 0.3  | 170.5 ± 19.7 | 121.8 ± 1.5     |
| V66E       | 57.7 ± 7.3  | 223.9 ± 7.6 | 143.8 ± 7.1       |
| V138M      | 67.7 ± 2.3  | 165.4 ± 4.5 | 149.3 ± 8.3        |
| I88F       | 75.3 ± 5.3  | 197.1 ± 9.7 | 156.4 ± 10.7       |
| N136D      | 81.4 ± 12.4 | 232.9 ± 3.9 | 149.4 ± 19.5       |
| M137K      | 86.3 ± 8.7  | 240.5 ± 0.5 | 161 ± 9.3         |
| Y81H       | 93.9 ± 2.2  | 128.5 ± 0.6 | 798.8 ± 1.3        |
| V171A      | 99.4 ± 9.9  | 155.8 ± 8.9 | 71.2 ± 10.5        |
| F21S       | 105.5 ± 3.2 | 189.3 ± 11.3 | 167.2 ± 9.8       |
| E234W      | 110.2 ± 3.1 | 194.7 ± 2.5 | 110.8 ± 7.4        |
| D311E      | 110.9 ± 1.2 | 175.4 ± 20.4 | 128.3 ± 10.6      |
| N26I       | 129.2 ± 5.7 | 172.3 ± 7.9 | 115.1 ± 18.1       |
| R232W      | 130.2 ± 5.1 | 167.5 ± 2.9 | 118.9 ± 3.2        |
| V155F      | 131.8 ± 14  | 189.3 ± 11.3 | 160 ± 1.8         |
| R232L      | 134.8 ± 8   | 191.6 ± 8.6 | 162.1 ± 0.9        |
| K37E       | 197.5 ± 18  | 253.8 ± 6.7 | 148.5 ± 11.4       |

FIGURE 2. Structural mutations of S. Typhimurium FimHSL1344 generated by random mutagenesis. The amino acid residues of S. Typhimurium FimHSL1344 are shown with gray highlights. The mannose binding-enhancing substitutions are marked in magenta; neutral substitutions are marked in light blue.
nose-binding pocket (Fig. 4, A and B, yellow). Thus, the homology of the predicted secondary structures of S. Typhimurium FimH and E. coli FimH allowed prediction of the tertiary structure of the former that, based on the location of the residue critical for mannose binding, is supported by previous experimental studies.

Distinction of Binding-enhancing Mutations in the Predicted Structure of S. Typhimurium FimH—Availability of the structural model of S. Typhimurium FimH allowed for the analysis of distribution of the functional mutations along the regions of predicted domains of the adhesin. To make the structural analysis more informative, we mapped the binding-enhancing and neutral mutations onto the domain-interacting configuration of S. Typhimurium FimH (as in Fig. 4B), where the position of mutations could be evaluated relative not only to the predicted binding pocket but also to the domain interaction interface. The putative mannose-binding pocket residues were defined by Phyre pocket/cleft predictive analysis, whereas the interdomain interface residues were determined using PyMOL directly from the corresponding structural model.

Most of the binding-enhancing mutations (13 of 17) mapped to the lectin domain, whereas only 9 of 24 functionally neutral mutations were positioned there. Although one of the binding-enhancing mutations, Y15F, was positioned in the putative mannose-binding pocket, most were located away from the pocket (Fig. 5A). Most functional mutations involved residues positioned close to or within the interdomain interface (Fig. 5, A and C). This was in contrast to the neutral mutations that were not as commonly found close to the interdomain interface (p < 0.05).

Four binding-enhancing mutations targeted three residues in the pilin domain (R232L/W, E234W, and D311E). One of them (D311E) was located within the interdomain interface, whereas two other mutations were located close to the interface on the β-strand, forming a sheet with the β-strand in the interface region (Fig. 5B). Although most of the neutral mutations were distributed throughout the pilin domain, two (E178A and T221A) were located in the interdomain interface.
Thus, mutations that enhance mannose-binding of *Salmonella* FimH tend to be located away from the predicted mannose-binding pocket and, instead, close to the putative interdomain interacting interface or even in the non-binding pilin domain. This suggests that the effect of the mutations on mannose-binding is indirect and thus allosteric in nature.

Shear-dependent Binding of *S. Typhimurium* FimH—The functional effects and structural distribution of the *S. Typhimurium* FimH mutations are highly similar to the pattern observed with binding-enhancing mutations in *E. coli* FimH that promote domain separation. In *E. coli* FimH, domain separation is associated with a shift from the low to high affinity binding state of the lectin domain (i.e. from the domain-interacting twisted conformation to the non-interacting untwisted conformation). The conformational switch is the basis of shear-dependent, allosteric catch bond properties of *E. coli* FimH, where application of the tensile force results in domain separation and, thus, strengthening of FimH binding to mannose. If, by analogy with *E. coli* FimH, *Salmonella* FimH also acts as an allosteric catch bond-forming adhesin, then *S. Typhimurium* FimH binding to mannosylated substrates should be enhanced under shear stress.

Above, we described mannose-BSA and RNase B binding by *S. Typhimurium* FimH under static conditions (i.e. without agitation- or flow-induced shear). Now, binding of the different *S. Typhimurium* FimH variants was tested in parallel plate flow chamber experiments, where bacteria were passed with different flow velocities over a surface coated with mannose-BSA or RNase B. As shown in Fig. 6A and B, at the lowest shear applied (0.01 piconewton/μm²), the relative difference in the levels of binding mediated by the low binding FimHSL1344 and high binding FimHLB5010 variants were very similar to those under static conditions. However, the low binding FimHSL1344 variant mediated 2-fold higher binding to mannose-BSA and RNase B when shear stress was increased 5-fold (to 0.05 piconewton/μm²) (Fig. 6A and B). The shear-enhanced binding was also observed for another low binding FimH variant (with neutral mutation S122T) when tested under increasing shear conditions on an RNase B-coated surface (Fig. 6B). In contrast, the high binding FimHLB5010 variant steadily decreased with increasing shear (Fig. 6A and B). The shear-inhibitable (shear-independent) binding pattern was characteristic for bacteria expressing FimH that carried the mutations increasing mannose binding (G39A, N136Y, and D311E, respectively). Furthermore, at the lowest shear, more than 80% of the FimHLB5010 bacteria were adhering in a firm (stationary) manner, but only about half of FimHSL1344 bacteria did so. The remainder of the bacteria was adhering rather weakly, resulting in bacterial rolling over the surface along the direction of flow (Fig. 6C and D).
With increasing shear, more and more of the rolling bacteria became firmly attached to the surface.

Thus, the FimH variants, which adhered relatively weakly under static conditions, displayed significantly enhanced binding under increased shear, indicating that the *S. Typhimurium* FimH adhesin displays shear-enhanced (shear-dependent) binding similar to *E. coli* FimH. In contrast, the *S. Typhimurium* FimH mutants that bind relatively strongly under static conditions appear to have lost the shear-dependent binding properties and instead mediate shear-independent binding that is rather inhibited by shear stress.

**Mannose-binding Capability of S. Typhimurium FimH Is Increased by Anti-FimH Antibody**—The shear-dependent binding properties of *S. Typhimurium* FimH and its structural homology to the *E. coli* FimH suggest that the putative interaction and separation of pilin and lectin domains could be the basis of a shift between low and high affinity conformations, respectively, of the *S. Typhimurium* adhesin. In *E. coli*, besides mechanical force (shear) and point mutations, an increase in binding affinity was also shown to be allosterically induced by interdomain wedging with monoclonal antibodies against an epitope within the interdomain interface (26). Here, we tested whether polyclonal antibodies against *Salmonella* FimH have a similar effect.

First, we analyzed binding of mannosylated HRP to purified type 1 fimbriae of *S. Typhimurium* in the presence and absence of antibodies raised against full-length *Salmonella* FimH. As shown in Fig. 7A, co-incubation of surface-immobilized *S. Typhimurium* fimbriae with anti-FimH antibodies resulted in a significant increase of HRP binding to fimbriae with the low binding (shear-dependent) FimHSL1344 variant and, to a much lesser extent, to fimbriae with the high binding (shear-independent) FimHAlb3 variant. In both cases, HRP binding was fully inhibited by α-mannosidase (Fig. 7A). Similar results were obtained when fimbriae were first pretreated with anti-FimH antibodies, which were then removed by washing before the addition of HRP (data not shown). In contrast, non-immune serum that does not recognize *Salmonella* FimH had no binding-increasing effect on HRP binding to the fimbriae.

The binding-activating effect of anti-FimH antibodies was also observed when *S. Typhimurium* adhesion to Hep-2 cells was tested (Fig. 7B). In regular medium conditions, the isogenic *S. Typhimurium* expressing the low binding FimHSL1344 (or low binding FimHS122T and FimHP301L with neutral mutations S122T and P301L, respectively) mediated low mannosidependent adhesion to Hep-2 cells (13.3 ± 0.6 x 10^3 and 5 ± 0.5 x 10^3 FimHSL1344-bacteria, 9.5 ± 5 x 10^3 and 3.3 ± 3 x 10^3 FimHS122T-bacteria, and 23.3 ± 6 x 10^3 and 1 ± 0 x 10^3 FimHP301L-bacteria adhered to Hep-2 monolayers (in one well of a 96-well plate), mean ± S.E. in the absence and presence of α-mannosidependent adhesion to Hep-2 cells (13.3 ± 0.6 x 10^3 and 5 ± 0.5 x 10^3 FimHSL1344-bacteria, 9.5 ± 5 x 10^3 and 3.3 ± 3 x 10^3 FimHS122T-bacteria, and 23.3 ± 6 x 10^3 and 1 ± 0 x 10^3 FimHP301L-bacteria adhered to Hep-2 monolayers (in one well of a 96-well plate), mean ± S.E. in the absence and presence of α-mannosidependent adhesion to Hep-2 cells (13.3 ± 0.6 x 10^3 and 5 ± 0.5 x 10^3 FimHSL1344-bacteria, 9.5 ± 5 x 10^3 and 3.3 ± 3 x 10^3 FimHS122T-bacteria, and 23.3 ± 6 x 10^3 and 1 ± 0 x 10^3 FimHP301L-bacteria adhered to Hep-2 monolayers (in one well of a 96-well plate), mean ± S.E. in the absence and presence of α-mannosidase (Fig. 7A). Similar results were obtained when fimbriae were first pretreated with anti-FimH antibodies, which were then removed by washing before the addition of HRP (data not shown). In contrast, non-immune serum that does not recognize *Salmonella* FimH had no binding-increasing effect on HRP binding to the fimbriae.

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the presence of anti-Salmonella antigen O group B serum or preimmune serum. In contrast, bacteria expressing the high binding FimH variants (FimH\(^{AJB3}\) and FimH\(^{D311E}\) that carry mannose-binding-increasing mutations N136Y and D311E, respectively) bound to Hep-2 cells at similar (high) levels under all tested conditions. Thus, the enhancement of the S. Typhimurium FimH binding by anti-FimH antibodies provides additional evidence that this adhesin has allosteric, catch bond-forming conformational properties similar to E. coli FimH.

**DISCUSSION**

Although S. enterica FimH and E. coli FimH belong to the same family of type 1 fimbrial adhesins, these two proteins have been shown to be evolutionarily distinct (non-orthologous) and share only little sequence identity (6, 7, 11). In fact, E. coli has a separate Salmonella-like fimbrial locus (sfm) that is 70% homologous to the S. enterica fim gene cluster, but its function has not yet been elucidated (7, 36). In the present study, we demonstrate that despite the lack of homology in primary sequence, S. enterica FimH represents a structural homolog of E. coli FimH with two-domain topology. We also show that S. enterica FimH exhibits shear-enhanced binding that, similar to E. coli, involves an allosteric two-domain regulatory mechanism and formation of catch bonds.

E. coli FimH is the most studied type-1 fimbrial adhesin among the Enterobacteriaceae. This two-domain protein has been shown to undergo conformational changes that allosterically regulate affinity to mannose (22, 26, 37). In native equilibrium (no shear force) conditions, the lectin and the pilin domains tightly interact with each other, and the former exhibits low affinity to its ligand. The low affinity state of the binding pocket is due to wide separation between mannose-binding loops when the lectin domain assumes a relatively more twisted and compressed conformation of the \(\beta\)-sandwich-like tertiary structure. However, separation of the domains results in an elongated untwisted conformation of the lectin domain and its shift to a high affinity state, with a finger trap-like closing of the mannose-binding pocket. The conformational changes provide the mechanism for shear-enhanced adhesion mediated by FimH under flow conditions. In this case, the shift from low affinity to high affinity conformation is initiated (and maintained) by mechanical force (shear force) physically separating the two domains (22, 38). Our studies on S. Typhimurium FimH indicate that Salmonella FimH, like E. coli FimH, exhibits shear-enhanced adhesion. We used naturally occurring FimH variants that were shown to mediate either relatively low or high affinity binding to eukaryotic cells under static conditions and also newly characterized FimH mutants obtained by random mutagenesis. In using the model mannosylated substrates in flow chamber experiments, 2- and 3-fold higher binding was mediated by the low binding FimH variants (FimH\(^{SL1344}\) and FimH\(^{H9252}\)) when shear stress was increased by 5-fold, from 0.01 to 0.05 piconewton/\(\mu\)m\(^2\). Moreover, increased shear substantially changed the mode of FimH\(^{SL1344}\) mediated adhesion, where fewer rolling (weakly adherent) bacteria than stationary (strongly adherent) bacteria were observed under high shear. In contrast, high binding variants of S. Typhimurium FimH (FimH\(^{H11021}\), FimH\(^{AJB3}\), or FimH\(^{D311E}\)) that differ from FimH\(^{SL1344}\) by G39A/F96S, N136Y, or D311E amino acid substitutions, respectively, exhibited shear-independent binding under flow conditions. This result is in agreement with previous reports that the FimH phenotype can be converted to shear independence by single point mutations (38). On the other hand, it has been also demonstrated that fimbrial shaft properties affect the binding pattern of the tip-associated FimH adhesin (39, 40). Although the exact mechanism of the shaft influence remains to be elucidated, it was proposed that the uncoiling/recouling ability of the main fimbrial stalk (41, 42) or differential flexibility of the minor ancillary subunits of the fimbrial tip (43) could have a profound effect on bacterial adhesion under dynamic fluidic conditions. Here, however, all tested FimH variants were expressed in an isogenic recombinant system with the same fimbrial shaft composition, so we can exclude potential impact of the major and auxiliary fimbrial components on

**FIGURE 7. Effect of anti-FimH antibodies on binding properties of S. Typhimurium FimH.** A, HRP binding to immobilized type 1 fimbriae (with FimH\(^{SL1344}\) or FimH\(^{AJB3}\)) in the absence or presence of anti-FimH antibody (1:2000 dilution) or preimmune serum (1:2000 dilution). B, binding of bacteria expressing different variants of FimH to Hep2-cells in the absence or presence of anti-FimH antibody (1:1000 dilution), anti-Salmonella antigen O group B serum (1:100 and 1:20 dilutions), or preimmune serum (1:100). Data are the means ± S.E. (error bars) of triplicates from one representative experiment of three experiments that were performed. Asterisks show the statistical significance of differences between indicated pairs of data sets: ***, \(p < 0.001\); **, \(p < 0.005\).
the observed binding properties of FimH. Nevertheless, it remains possible that FimH variants analyzed here may display altered properties of binding when expressed on different Salmonella type 1 fimbrial shafts. The shear dependence of binding has been recently demonstrated for other fimbrial adhesins, including the type-1 fimbrial adhesin FimH of Klebsiella pneumoniae (an evolutionary orthologue of E. coli FimH) and the genetically and functionally distinct adhesins PapGII of P-fimbriae and CfaE from CFA/I fimbriae of E. coli (44–46). Structural analyses have shown that all of these proteins are composed of two domains, a fimbria-anchoring pilin domain and an apex-positioned binding domain. For the CfaE adhesin, it was further demonstrated that interdomain interactions allosterically regulate binding affinity. Therefore, we hypothesized that a similar allosteric mechanism could be involved in shear-dependent properties of S. Typhimurium FimH. Because the tertiary structure of Salmonella FimH has not yet been solved, we performed in silico modeling of S. Typhimurium FimH using fold recognition and template modeling software (30–33). Our analysis revealed that S. Typhimurium FimH, despite the lack of homology in primary sequence, is likely to be a structural homologue of E. coli FimH, displaying a remarkable homology of secondary structure. Thus, the model S. Typhimurium FimH consists of two domains, each containing a cysteine bridge in a similar location as in E. coli. Disulfide bonds are considered to be critical for proper protein folding, stability, and function (47). In adhesive proteins, disulfide bonds are often located near binding sites, and their importance in optimizing receptor-ligand interactions has been demonstrated (48–50). In E. coli FimH, it has been shown that a disulfide bond located near the binding pocket, although having no effect on binding under static conditions, is critical for adhesion under shear forces (51), and its removal from the lectin domain drastically decreased adhesion under high fluid flow.

We obtained two putative models of Salmonella FimH based on the high affinity and low affinity conformations of E. coli FimH, where the lectin domain is separated from or closely interacting with the pilin domain, respectively. In our models, the lectin domain takes an elongated shape with a tight binding pocket in the high affinity conformation and a twisted shape with a loose binding pocket in the low affinity conformation.

General suitability of the models is supported by the fact that the threonine residue (Thr-56) that is critical for mannose binding in Salmonella FimH (35) mapped to the top loops of the N-terminal domain exactly where the mannose-binding pocket is predicted to be located. Additional support for the model comes from the similarity of the distribution pattern of binding-enhancing mutations along the tertiary structure of Salmonella FimH with the pattern shown for E. coli FimH. As in E. coli, mutations that increase mannose binding under static conditions are positioned mostly within or close to the interdomain interface, with some located in the pilin domain. By analogy with E. coli, we propose that these mutations interfere with the domain-domain interaction, thus promoting separation of the domains. In turn, this allows the lectin domain to switch from a low to high affinity conformation even under static (no shear) conditions. Thus, taken together, the structure-functional analysis demonstrates that S. Typhimurium FimH is very similar to FimH of E. coli and displays shear-enhanced adhesion by a mechanism involving allosteric connections between the interdomain interface and the mannose-binding pocket.

The catch bond properties of evolutionarily distinct proteins indicate the importance of such binding properties for bacterial interactions with the host. It was demonstrated that physiological advantages of allosteric catch bond adhesion include resistance to soluble inhibitors and the ability to rapidly spread along the colonization surface (44, 52). On the other hand, similar to Salmonella FimH, naturally occurring point mutations that increase affinity to mannose under low shear are also found in E. coli FimH (24, 38, 53). These mutations are positively selected in uropathogenic E. coli strains and increase urotopism and urinary tract colonization properties of the bacteria (23), possibly because under lower shear stress in the bladder and/or kidneys and in lower concentrations of soluble mannosylated compounds in urine, the catch bond mechanism of bacterial adhesion may not be advantageous. Although binding-enhancing mutations in S. Typhimurium enhance cell tropism, biofilm formation in the intestine and likely intracellular spread of the bacteria (14, 18), the effects of the mutations on Salmonella virulence and any selective advantage they may provide in nature remain undefined. To answer these questions, a detailed experimental and populational study of FimH variations is required.

Here we also describe another phenomenon that is potentially relevant to the physiological role of Salmonella FimH. We found that anti-FimH antibodies do not inhibit the mannose-binding capability of the adhesin but instead enhance it. Anti-Salmonella FimH antibodies raised against full-length FimH with a C-terminal His tag recognize fimbrial FimH (supplemental Fig. S3) and isolated lectin domain of S. Typhimurium in the Western blot (not shown), but reactivity with the pilin domain has not been tested. Although the lack of inhibitory activity of the antibodies could be explained by poor immunogenicity of the mannose-binding loops of FimH, the binding-activating property is likely to be associated with the allosteric multiconformational nature of the adhesin. Monoclonal antibodies with similar effect toward E. coli FimH have been reported previously (26). These antibodies (clone MAb21) specifically recognize the high affinity conformation of the E. coli FimH lectin domain and enhance mannose binding by preventing FimH from shifting back to the low affinity conformation. In both E. coli and Salmonella studies, the enhancement is not likely to be due to bacterial or fimbrial aggregation by the antibodies because in the HRP binding test, the isolated fimbriae treated with anti-FimH antibodies were not in solution but immobilized (well below saturation) on the plastic surface. Also, in the case of MAb21 (26) and anti-Salmonella FimH antibodies, the same binding-activating effect was observed in the presence of purified Fab fragments. The MAb21-specific epitope is located in the interdomain region of FimH, and, thus, the stabilization of the high affinity conformation could be due to the antibodies wedging between the domains. Similarly, the binding-activating effect of the antibodies against the Salmonella FimH may be due to facilitation of the high affinity conformation of the adhe-
sin via interdomain wedging by the antibodies specific to either lectin or pilin domain. The key difference with the *E. coli* studies, however, is that here we used polyclonal antibodies raised against the entire FimH rather than monoclonal antibody clone against purified lectin domain. Also, the anti-FimH antibodies bound equally well to the high binding and low binding *S. Typhimurium* FimH variants, whereas the MAb21 antibodies recognized preferentially the high binding variants of *E. coli* FimH (26). It is likely that, besides the binding-activating antibodies, a large portion of the polyclonal antibodies does not differentiate between specific FimH conformations.

Although the molecular details of *Salmonella* FimH interaction with different specific antibodies remain to be determined, it is clear that epitopes inducing the binding-enhancing response are highly immunogenic. This could become an issue for development of FimH-based vaccines, especially considering that the mannose-binding loops may not induce a strong immune response. The *E. coli* FimH-lectin or its lectin domain has been a target for vaccine development to protect against urinary tract infection (54–56). Although protective effects have been reported in mice and primates, the mechanism of the protection has not been investigated, and these studies did not lead to any reported successes in human trials. The induction of an adhesion-enhancing polyclonal antibody response is also seen for *E. coli* FimH (57) and could be a factor complicating vaccine development. Also, it remains to be determined whether the natural immune response to *E. coli* or *Salmonella* infections results in anti-FimH antibodies with binding-activating properties and, if so, whether this could be of any benefit to the pathogen (or the host). One way or another, it appears that besides the binding properties *per se*, function-modulating effects of a conformation-specific immune response could also be a physiologically significant phenomenon to be considered in studies of the increasing number of bacterial adhesins that are found to form allosteric catch bonds.

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REFERENCES

1. Ofek, I., Mirelman, D., and Sharon, N. (1977) *Nature* **265**, 623–625
2. Bhattacharjee, J. W., and Srivastava, B. S. (1978) *J. Gen. Microbiol.* **107**, 407–410
3. Duguid, J. P., and Old, D. C. (1980) in *Bacterial Adherence* (Beachey, E. H., ed) pp. 185–217, Chapman and Hall, London
4. Imberty, A., Wimmerová, M., Mitchell, E. P., and Gilboa-Garner, N. (2004) *Microbes Infect.* **6**, 221–228
5. Pretzer, G., Snel, J., Molenaar, D., Wiersma, A., Bron, P. A., Lamberts, J., de Vos, W. M., van der Meer, R., Smits, M. A., and Kleerebezem, M. (2005) *J. Bacteriol.* **187**, 6128–6136
6. Boyd, E. F., and Hartl, D. L. (1999) *J. Bacteriol.* **181**, 1301–1308
7. Nuccio, S. P., and Bäumler, A. J. (2007) *Microbiol. Mol. Biol. Rev.* **71**, 551–575
8. Clegg, S., Hull, S., Hull, R., and Pruckler, J. (1985) *Infect. Immun.* **48**, 275–279
9. Gerlach, G. F., Clegg, S., Ness, N. J., Swenson, D. L., Allen, B. L., and Nichols, W. A. (1989) *Infect. Immun.* **57**, 764–770
10. Korhonen, T. K., Lounatmaa, K., Ranta, H., and Kuusi, N. (1980) *J. Bacteriol.* **144**, 800–805
11. Thankavel, K., Shah, A. H., Cohen, M. S., Ikeda, T., Lorenz, R. G., Curtiss, R., and Abraham, S. N. (1999) *J. Biol. Chem.* **274**, 5797–5809
12. Collinson, S. K., Liu, S. L., Clouthier, S. C., Banser, P. A., Doran, J. L., Anderson, K. E., and Kay, W. W. (1996) *Gene* **169**, 75–80
13. Bäumler, A. J., Tsolis, R. M., and Heffron, F. (1997) *Adv. Exp. Med. Biol.* **412**, 149–158
14. Boddicker, J. D., Ledebør, N. A., Jagnow, J., Jones, B. D., and Clegg, S. (2002) *Mol. Microbiol.* **45**, 1255–1265
15. Ewen, S. W., Naughton, P. J., Grant, G., Sojka, M., Allen-Vercoe, E., Bar-rod, S., Thrors, C. J., and Pusztai, A. (1997) *FEBS Lett.* **412**, 185–192
16. Althouse, C., Patterson, S., Fedorka-Cray, P., and Isaacsen, R. E. (2003) *Infect. Immun.* **71**, 6446–6452
17. Naughton, P. J., Grant, G., Baro, S., Allen-Vercoe, E., Woodward, M. J., and Pusztai, A. (2001) *J. Med. Microbiol.* **50**, 191–197
18. Guo, A., Cao, S., Tu, L., Chen, P., Zhang, C., Jia, A., Yang, W., Liu, Z., Chen, H., and Schifferli, D. M. (2009) *Microbiology* **155**, 1623–1633
19. Guo, A., Lasaro, M. A., Sirard, J. C., Krolewski, J., and Schifferli, D. M. (2008) *Microbiology* **153**, 1059–1069
20. Choudhury, D., Thompson, A., Stojanoff, V., Langermann, S., Pinkner, J., Hultgren, S. J., and Knight, S. D. (1999) *Science* **285**, 1061–1066
21. Hung, C. S., Boutskaer, J., Hung, D., Pinkner, J., Widberg, C., De Fusco, A., Krogfelt, K. A., Trintchina, E., Vogel, V., Thomas, W., and Sokurenko, E. (2007) *J. Biol. Chem.* **282**, 23437–23446
22. Trintchina, E., Trintchina, A., Forero, M., Vogel, V., Thomas, W., and Sokurenko, E. (2008) *J. Biol. Chem.* **283**, 7823–7833
23. Hancock, J. S., Yeh, K. S., and Clegg, S. (1997) *FEBS Lett.* **412**, 289–296
24. Sambrook, J., and Russell, D. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
25. Thomas, W. E., Nilsson, L. M., Forero, M., Sokurenko, E. V., and Vogel, V. (2004) *Mol. Microbiol.* **53**, 1545–1557
26. Bennett-Lovsey, R. M., and Sternberg, M. J., and Kelley, L. A. (2008) *Proteins* **70**, 611–625
27. Roy, A., Kucukural, A., and Zhang, Y. (2010) *Nat. Protoc.* **5**, 725–738
28. Arnold, K., Bordoli, L., Kopp, I., and Schwede, T. (2006) *Bioinformatics* **22**, 195–201
29. Esvar, N., Webb, B., Marti-Renom, M. A., Madhusudhan, M. S., Eramian, D., Shen, M. Y., Pieper, U., and Sahi, S. (2006) *Current Protocols in Bioinformatics*, Chapter 5, Unit 5.6
30. Kisiela, D., Sapeta, A., Kuczowski, M., Stefanik, T., Wieliczko, A., and Ugorski, M. (2005) *Infect. Immun.* **73**, 6187–6190
31. Korea, C. G., Badoura, R., Prevost, M. C., Ghigo, J. M., and Beloin, C. (2010) *Environ. Microbiol.* **12**, 1957–1977
32. Yakovenko, O., Sharma, S., Forero, M., Tchesnokova, V., Aprikian, P., Kidd, B., Mach, A., Vogel, V., Sokurenko, E., and Thomas, W. E. (2008) *J. Biol. Chem.* **283**, 11596–11605
33. Thomas, W. E., Trintchina, E., Forero, M., Vogel, V., and Sokurenko, E. (2002) *Cell* **109**, 913–923
34. Madison, B., Ofek, I., Clegg, S., and Abraham, S. N. (1994) *Infect. Immun.* **62**, 843–848
35. Duncan, M. J., Mann, E. L., Cohen, M. S., Ofek, I., Sharon, N., and Abra-
ham, S. N. (2005) J. Biol. Chem. 280, 37707–37716
41. Forero, M., Yakovenko, O., Sokurenko, E. V., Thomas, W. E., and Vogel, V. (2006) PLoS Biol. 4, e298
42. Bullitt, E., and Makowski, L. (1998) Biophys. J. 74, 623–632
43. Aprikian, P., Interlandi, G., Kidd, B. A., Le Trong, I., Tchesnokova, V., Yakovenko, O., Whitfield, M. J., Bullitt, E., Stenkamp, R. E., Thomas, W. E., and Sokurenko, E. V. (2011) PLoS Biol. 9, e1000617
44. Nilsson, L. M., Thomas, W. E., Sokurenko, E. V., and Vogel, V. (2006) Appl. Environ. Microbiol. 72, 3005–3010
45. Stahlhut, S. G., Tchesnokova, V., Struve, C., Weissman, S. J., Chattopadhyay, S., Yakovenko, O., Aprikian, P., Sokurenko, E. V., and Krogfelt, K. A. (2009) J. Bacteriol. 191, 6592–6601
46. Tchesnokova, V., McVeigh, A. L., Kidd, B., Yakovenko, O., Thomas, W. E., Sokurenko, E. V., and Savarino, S. J. (2010) Mol. Microbiol. 76, 489–502
47. Sevier, C. S., and Kaiser, C. A. (2006) Antioxid. Redox Signal. 8, 797–811
48. Sung, Y. H., Hong, H. D., Cheong, C., Kim, J. H., Cho, J. M., Kim, Y. R., and Lee, W. (2001) J. Biol. Chem. 276, 44229–44238
49. Merckel, M. C., Tanskanen, J., Edelman, S., Westerlund-Wikström, B., Korhonen, T. K., and Goldman, A. (2003) J. Mol. Biol. 331, 897–905
50. Pettigrew, D., Anderson, K. L., Billington, J., Kota, E., Simpson, P., Urvil, P., Rabuzin, F., Roversi, P., Nowicki, B., du Merle, L., Le Bouguenec, C., Matthews, S., and Lea, S. M. (2004) J. Biol. Chem. 279, 46851–46857
51. Nilsson, L. M., Yakovenko, O., Tchesnokova, V., Thomas, W. E., Schembri, M. A., Vogel, V., Klemm, P., and Sokurenko, E. V. (2007) Mol. Microbiol. 65, 1158–1169
52. Anderson, B. N., Ding, A. M., Nilsson, L. M., Kusuma, K., Tchesnokova, V., Vogel, V., Sokurenko, E. V., and Thomas, W. E. (2007) J. Bacteriol. 189, 1794–1802
53. Sokurenko, E. V., Chesnokova, V., Doyle, R. J., and Hasty, D. L. (1997) J. Biol. Chem. 272, 17880–17886
54. Langermann, S., and Ballou, W. R. (2003) Adv. Exp. Med. Biol. 539, 635–648
55. Langermann, S., Möllby, R., Burlein, J. E., Palaszynski, S. R., Auguste, C. G., Defusco, A., Strouse, R., Schenerman, M. A., Hultgren, S. J., Pinkner, J. S., Winberg, J., Guldevall, L., Söderhäll, M., Ishikawa, K., Normark, S., and Koenig, S. (2000) J. Infect. Dis. 181, 774–778
56. Poggio, T. V., La Torre, J. L., and Scodeller, E. A. (2006) Can. J. Microbiol. 52, 1093–1102
57. Tchesnokova, V., Aprikian, P., Kisiela, D., Gowey, S., Korotkova, N., Thomas, W., and Sokurenko, E. (2011) Infect. Immun., in press