Supplementary Materials to:

Role of Extracellular Structures of *Escherichia coli* O157:H7 in Initial Attachment to Biotic and Abiotic Surfaces

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Table S1. Oligonucleotides used in this study

ID	Sequence	Use
ΔcsgA-F	ACAACGTTAATTTCCATTCGACTTTTAAATCAATCCGATGGGGGTTTTACGTGTAGGCTGGAGCTGCTTC	Mutagensis
ΔcsgA-R	CCCGAAAAAAAAACAGGGCTTGCGCCCTGTTTCTTTAATACAGATGATGTAATGGGAATTAGCCATGGTCC	Mutagensis
ΔespA-F	GACGTTACAGACAGGGTATCGTTATTTACGTTAAGCATAGTTATCTCCGGGTGTAGGCTGGAGCTGCTTC	Mutagensis
ΔespA-R	ACTCAATAATTTTTTTGTTTTCCTGAGAAAAATTATCAAGAGGTATATAGATGGGAATTAGCCATGGTCC	Mutagensis
ΔfliC-F	ATCAGGCAATTTGGCGTTGCCGTCAGTCTCAGTTAATCAGGTTACAACGAGTGTAGGCTGGAGCTGCTTC	Mutagensis
ΔfliC-R	GGTGGAAACCCAATACGTAATCAACGACTTGCAATATAGGATAACGAATCATGGGAATTAGCCATGGTCC	Mutagensis
ΔhcpA-F	TGATAACGCAGGCACAGGGCCGTGAGCTGTGGAATATTCATTGCCGCTCCGTGTAGGCTGGAGCTGCTTC	Mutagensis
ΔhcpA-R	GCTGATGGCGTTTGCCAAAGTAGCACCAACCAAATCAAGGAGCGAAACAGATGGGAATTAGCCATGGTCC	Mutagensis
ΔlpfA-F	ACTTTGACGACTAATGGCGGCAATTACGCCGCCATTTGTAAAACGGACGAGTGTAGGCTGGAGCTGCTTC	Mutagensis
ΔlpfA-R	AGTTGTCGATTTTAATACATCAAGATTTTCTTTTTAATGTAATTTTTTAAATGGGAATTAGCCATGGTCC	Mutagensis
ΔompA-F	AAAGGCAAAAAAAAACCCCGCAGCAGCGGGGTTTTTCTACCAGACGAGAACGTGTAGGCTGGAGCTGCTTC	Mutagensis
ΔompA-R	CTCGTTGGAGATATTCATGGCGTATTTTGGATGATAACGAGGCGCAAAAAATGGGAATTAGCCATGGTCC	Mutagensis
Δper-F	CCATCTGAATTCAACGCAATTTTCATGAATGACCTTTACAATATTTTAGGGTGTAGGCTGGAGCTGCTTC	Mutagensis
Δper-R	TATAAATGTAGTTTTAAAAAACATATCGATAGACAGTTAAATATAAGAGGATGGGAATTAGCCATGGTCC	Mutagensis
ΔyehD-F	ATGGCGGCCATTTTAACTTACTGAAAAAACAAGATGATTATTTTAAATATGTGTAGGCTGGAGCTGCTTC	Mutagensis
ΔyehD-R	AGAGATACAGACTCTTAACAAAACAAAATAAACTTATTAAGGAATGCATCATGGGAATTAGCCATGGTCC	Mutagensis
CTR-ΔcsgA-F	AACTTTTGGCGGTTGTTGC	Mutation confirmation
CTR-ΔespA-F	GACTCGAGACTTTCTGCG	Mutation confirmation
CTR-ΔfliC-F	CGTCATAGCGTTCGACGG	Mutation confirmation
CTR-ΔhcpA-F	CAGCGTGTTAACATCC	Mutation confirmation
CTR-ΔlpfA-F	GGTGTTATATTTAATTTTAG	Mutation confirmation
CTR-ΔompA-F	GTCAATCAAATGTGGC	Mutation confirmation
CTR-Δper-F	AGGTATATGCCCTAATATC	Mutation confirmation
CTR-ΔyehD-F	TGACGATAATCTCATATTTTCC	Mutation confirmation
CTR-Cm-R	GCAGGGCGGGGCGTAAGGCG	Mutation confirmation
pGFP-F	ACTCTTCCTTTTTCAATATTATTGAAGC	pGFP frame amplification
pGFP-R	CTGTCAGACCAAGTTTACTC	pGFP frame amplification
Gen-F	TTAGGTGGCGGTACTTGGG	Gen-R amplification
Gen-R	ATGTTACGCAGCAGCAACG	Gen-R amplification
mCherry-F	GATCCCCGGGTACCGGTAGAAAAAATGGTGAGCAAGGGCGAG	mCherry amplification
mCherry-R	TCAGTTGGAATTCTACGAATGTTACTTGTACAGCTCGTCCATG	mCherry amplification
pET-fliC-F	AGAAGGAGATATAAGATGGCACAAGTCATTAATACC	*fliC* amplification
pET-fliC-R	ATGGTGATGGTGATGTTAACCCTGCAGCAGAGAC	*fliC* amplification
pET-ompA-F	AGAAGGAGATATAAGATGAAAAAGACAGCTATCGC	*ompA* amplification
pET-ompA-R	ATGGTGATGGTGATGTTAAGCTTGCGGCTGAGTT	*ompA* amplification
pET-per-F	AGAAGGAGATATAAGATGAAAATGAAATATATACCAGT	*per* amplification
pET-per-R	ATGGTGATGGTGATGCTATTTATCACTATAAAATTCGT	*per* amplification

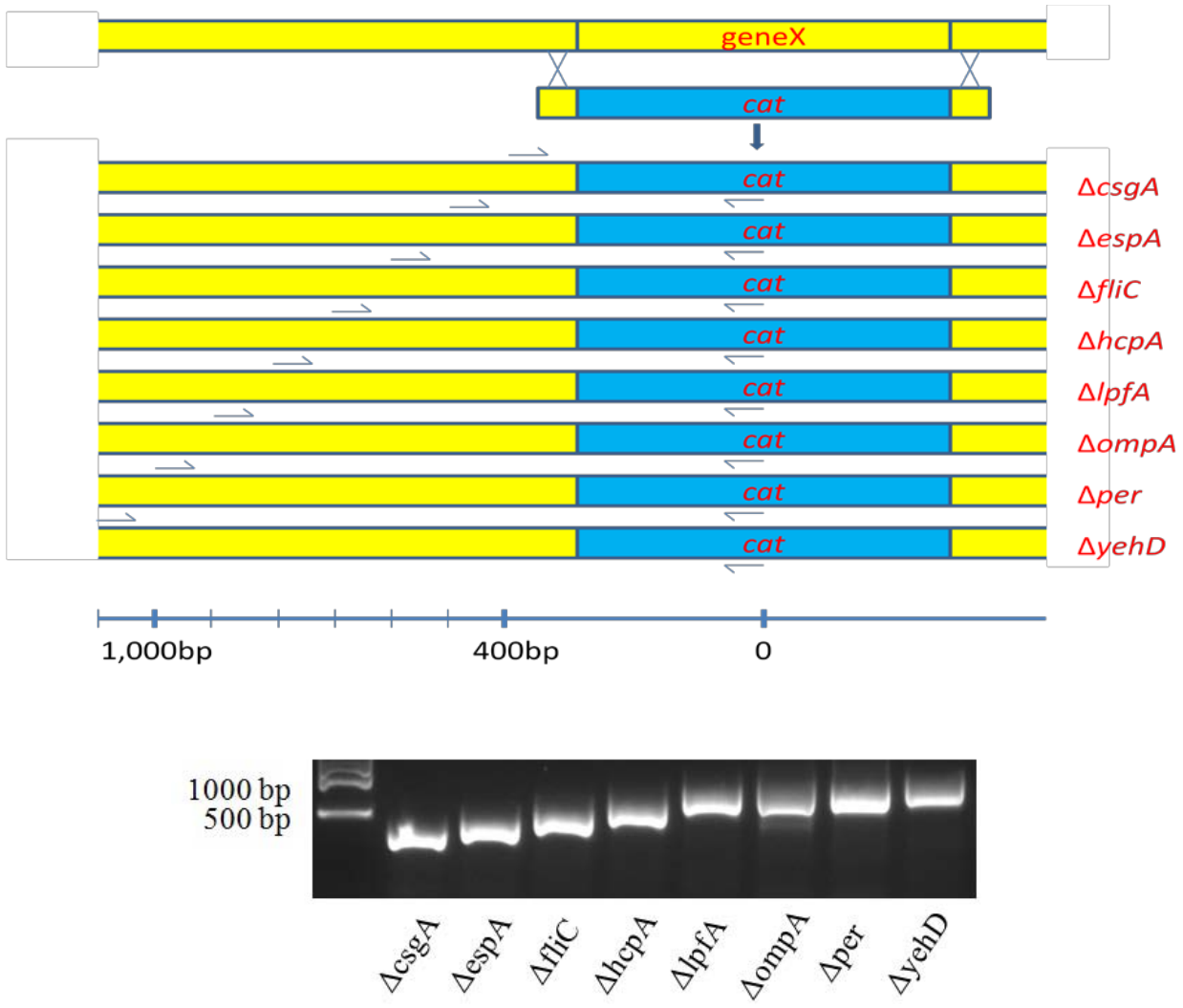

Figure S1. Mutant construction and confirmation

Top: Schematic presentation of the *cat* gene knock-in in the mutants and relative positions of the oligonucleotides (arrows) for PCR confirmation of the mutants. The ruler indicates expected size (400-1,100bp with 100 bp increments.) of PCR fragment encompassing the 5' deletion junction for each of the deleted genes. **Bottom:** Agarose gel showing the different sizes of PCR fragments using reverse primer (CTR-Cm-R) specific for the cat gene and forward primers (CTR-ΔcsgA-F, CTR-ΔespA-F, CTR-ΔfliC-F, CTR-ΔhcpA-F, CTR-ΔlpfA-F, CTR-ΔompA-F, CTR-Δper-F , and CTR-ΔyehD-F) specific for each of the intended mutants. Lane 1: Bio-Rad EZ Load 500 bp molecular ruler.

Methods: To generate the deletion mutant strains, the pKD46 plasmid was transformed into EDL933 strain by electroporation using a Gene Pulser Transfection Apparatus with a Pulse Controller (BioRad, Hercules, CA). A total of eight different genes were eliminated by using the λ Red recombination technology (*, see notes) in EDL933. EDL933Δ*csgA*, EDL933Δ*espA*, EDL933Δ*fliC*, EDL933Δ*hcpA*, EDL933Δ*lpfA*, EDL933Δ*ompA*, EDL933Δ*per* and EDL933Δ*yehD* were generated using amplification products obtained by PCR with primer pairs ΔcsgA-F/ΔcsgA-R, ΔespA-F/ΔespA-R, ΔfliC-F/ΔfliC-R, ΔhcpA-F/ΔhcpA-R, ΔlpfA-F/ΔlpfA-R, ΔompA-F/ΔompA-R, Δper-F/Δper-R *and* ΔyehD-F/ΔyehD-R (Integrated DNA Technologies,

Coralville, IA) and plasmid pKD3 as the template. The sequence of oligonucleotides is listed in Table S1. PCR products were generated using GoTaq Green master mix (Promega, Madison WI) and purified from 1% agarose gel using GeneJet Gel Extraction kit (Thermo Scientific). The purified DNA was suspended in elution buffer (1 mM EDTA, 10 mM Tris, pH 8.0).The oligonucleotide design and the following steps of mutant EDL933 strain generation was accomplished as described in Datsenko *et al* (*, see notes) and Serra-Moreno et al. **, see notes). Briefly, EDL933 transformants carrying the pKD46 plasmid were grown in 25 mL TSB cultures with ampicillin and L-arabinose at 30°C to an OD_{600} of ≈0.3 and then made electrocompetent by concentrating 100-fold and washing three times with ice-cold MilliQ water. Electroporation was done with a Gene Pulser Transfection Apparatus equipped with Pulse Controller according to the manufacturer's instructions (BioRad). 100 µl of cells and 10–100 ng of PCR product was electroporated in 0.1-0.2 mm electroporation cuvettes (BioRad). Shocked cells were added to 1 ml SOC, incubated 3 hours at 37°C, and then spread onto 5 µg/ml Cm LB plates to select Cm^R transformants.
The deletion of the gene of interest was verified with PCR, using oligonucleotides CTR-ΔcsgA-F, CTR-ΔespA-F, CTR-ΔfliC-F, CTR-ΔhcpA-F, CTR-ΔlpfA-F, CTR-ΔompA-F, CTR-Δper-F and CTR-ΔyehD-F, each paired with CTR-Cm-R, and cells from presumed mutant strains as DNA templates. The PCR products were analyzed on 1% agarose gel. The expected sizes of the PCR products ranged from 400 to 1100 bp, with 100 bp increments. The sequence of oligonucleotides is listed in Table S1.

Notes:

* **Datsenko KA, Wanner BL.** 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci U S A **97:**6640-6645.

** **Serra-Moreno R, Acosta S, Hernalsteens JP, Jofre J, Muniesa M.** 2006. Use of the lambda Red recombinase system to produce recombinant prophages carrying antibiotic resistance genes. BMC Mol Biol **7:**31.

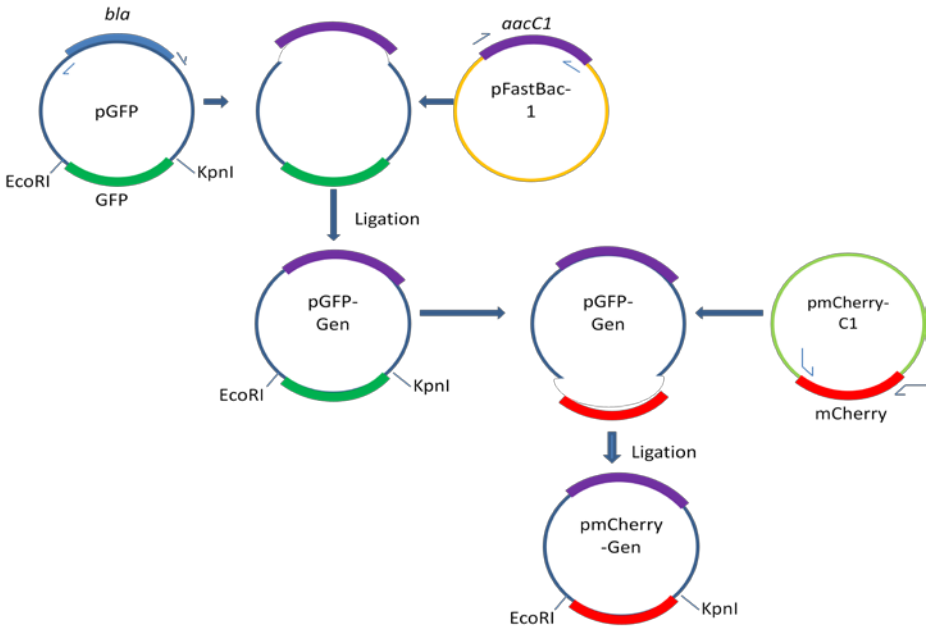

Figure S2. Schematic presentation of the construction of plasmids used for expressing green and red fluorescent proteins.
Ampicillin resistant gene (*bla*), gentamicin resistant gene (*aacC1*), GFP, and mCherry sequences are represented by ble, purple, green, and red segments. Arrows indicate the location and orientation of oligonucleotide annealing sites. Restriction sites used for DNA manipulation are labeled. Other features of the plasmids are not shown. Not drawn to scale.

Methods: To construct the gentamicin resistant pGFP plasmid (pGFP-Gen), two PCR products were made. First, using oligonucleotides pGFP-F/pGFP-R, and pGFP plasmid as a template, a linear double stranded DNA was amplified which contained the entire sequence of the pGFP plasmid except the coding sequence of ampicillin-resistance gene (*bla*). The second PCR product was prepared using oligonucleotides Gen-F/Gen-R, and pFastBac1 as template, to amplify the coding sequence of the gentamicin resistance gene (*aacC1*). The PCR products were generated using Phusion long range high fidelity DNA polymerase (Thermo Fisher Scientific, Pittsburgh, PA). The PCR product of *aacC1* gene was phosphorylated with T4 polynucleotide kinase and ligated to the PCR product of the pGFP frame using T4 DNA ligase (Promega). The ligation reaction (5 µl) was electroporated into Top10 cells (Life Technologies, Carslbad, CA). Transformant was selected on LB plate containing gentamicin.
The pmCherry-Gen^{R} plasmid was constructed using pGFP-Gen plasmid by replacing the GFP gene with the gene encoding mCherry from plasmid pmCherry-C1. The pGFP-Gen plasmid was digested with KpnI and EcoRI restriction endonucleases (Promega). The mCherry gene was amplified using oligonucleotide mCherry-F/mCherry-R using pmCherry-C1 as a template. The PCR product was digested with KpnI and EcoRI restriction endonucleases and ligated with the pGFP-Gen backbone. The ligation reaction (5 µl) was electroporated into Top10 cells. Transformant was selected on LB plate containing gentamicin. Plasmids were prepared from the respective Top 10 transformants and transferred into EDL 933 and the isogenic mutants by electroporation. The sequence of oligonucleotides is listed in Table S1.

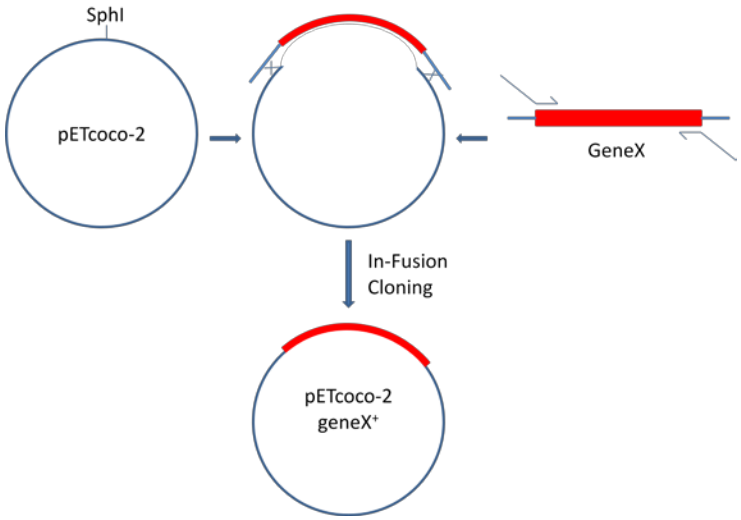

Figure S3. Schematic presentation of construction of plasmids used for complementation of selected mutations.

Methods: For complemented mutant experiments *fliC*, *ompA* and *per* genes were cloned into pETcoco-2 plasmid (Novagen, Billerica, MA) using In-Fusion HD EcoDry Cloning Plus kit (Clontech Laboratories, Mountain View, CA). The target gene inserts were prepared by PCR using oligonucleotides pairs pET-fliC-F/pET-fliC-R (*fliC* insert), pET-ompA-F/pET-ompA-R (*ompA* insert), and pET-per-F/pET-per-R (*per* insert), and EDL933 cells as DNA template. The PCR products are gel purified. The sequence of oligonucleotides is listed in Table S1. The plasmid pETcoco-2 was linearized with SphI (Thermo Fisher Scientific, Pittsburgh, PA) digestion. Linearized pETcoco-2 DNA is incubated with individual PCR products following the instructions of the In-fusion cloning kit. The final constructs were electroporated into Top10 as and Ampicillin resistant transformants are selected. The recombinant plasmids are extracted from the transformants following induction by L-arabinose (to increase plasmid copy numbers) and the presence of the complementation genes are confirmed by PCR using the primer pairs described above. The confirmed complementary plasmids are transformed into the corresponding mutant strains. The complemented strain (the mutants with the complementation plasmid) are confirmed by PCR reactions showing the correct insertion site of the Cm-resistant gene (*cat*) (Figure S1) and the presence of the complementation gene.

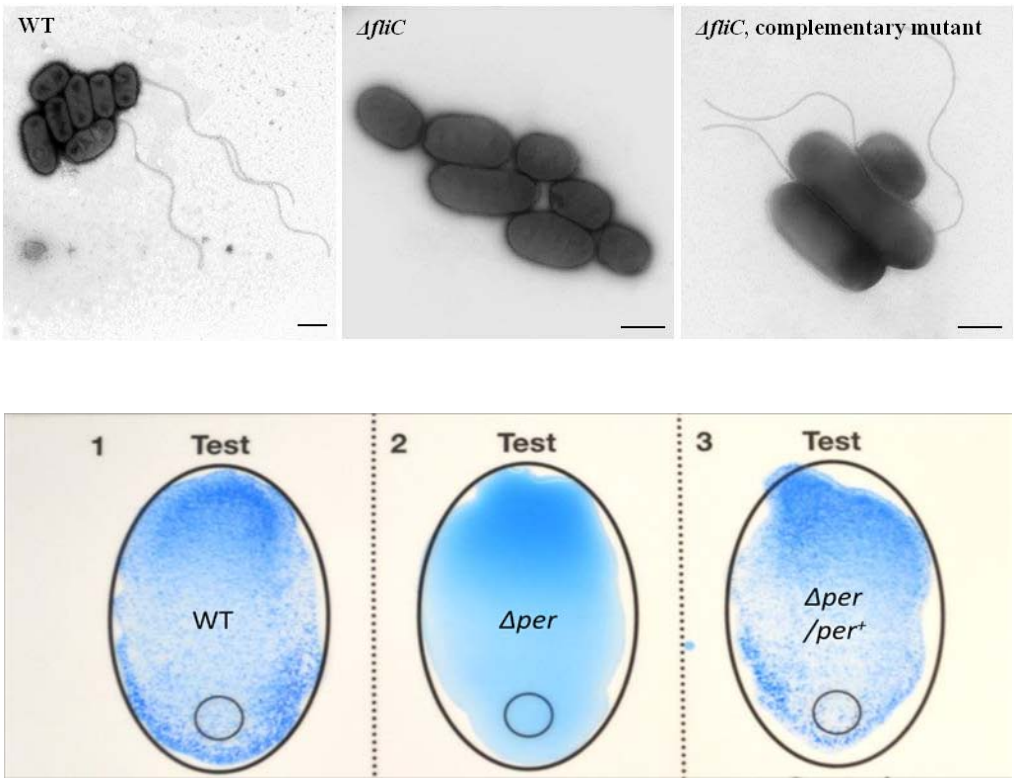

Figure S4. Morphological complementation of *ΔfliC* and *Δper* mutants pETcoco-2 complementary plasmids.
Top: Negative staining TEM showing flagella production in *E. coli* O157:H7 EDL933 wild type, *ΔfliC* mutant, and complemented *ΔfliC/ fliC*$^{+}$ strains. Scale bar: 500 nm. **Bottom:** Agglutination of *E. coli* O157:H7 EDL933 wild type, *Δper* mutant, and complemented *Δper/ per*$^{+}$ strains using Oxoid DrySpot O157 agglutination assay.

Methods: For negative staining SEM, bacterial cells suspended in growth medium (without fixation or centrifugation), and were applied directly onto 200 mesh formvar-coated copper grids and allowed to absorb for 1-2 minutes. Excess liquid was then wicked off with filter paper and grids were negatively stained with drops of 0.025% phosphotungstic acid (w/v). After staining for 5 seconds the excess stain was wicked off and grids were allowed to air dry. Samples were viewed and imaged at 80kV with a Hitachi HT-7700 transmission electron microscope (Hitachi, Tokyo, Japan).
Agglutination was carried out following manufacturer's instructions of Oxoid DrySpot O157 agglutination assay kit.

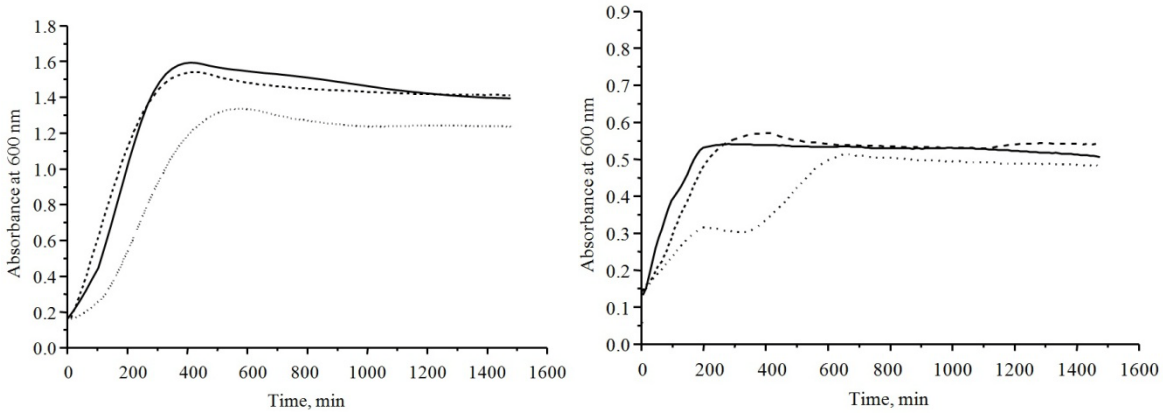

Figure S5. Complementation of growth characteristics of *Δper* and *ΔompA* mutants.
Left: Growth kinetics of *E. coli* O157:H7 EDL933 wild type (solid line), *Δper* mutant (light dotted line), and complemented *Δper/*pETcoco-2 *per*$^{+}$ (dark dotted line) strains in 1x TSB. **Right:** Growth kinetics of *E. coli* O157:H7 EDL933 wild type (solid line), *ΔompA* mutant (light dotted line), and complemented *ΔompA/*pETcoco-2 *ompA*$^{+}$ (dark dotted line) strains in 10% TSB.

Methods: Growth kinetics of individual strains was determined as described in the manuscript.

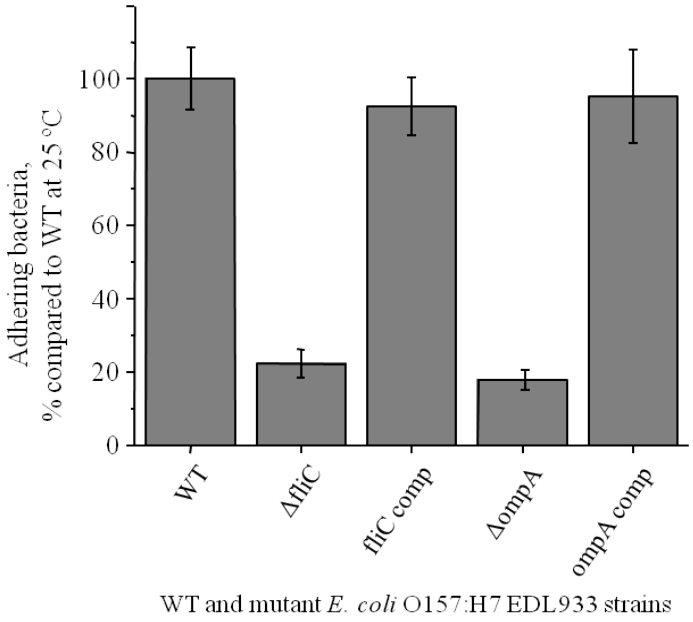

Figure S6. Adhesion of WT and mutant *E. coli* O157:H7 EDL933 strains on spinach.

Methods: WT, deletion mutants, and complementary mutants were incubated with spinach leaves at 25 ^{o}C for 24 h as described in the manuscript. The enumeration after the detachment showed that the complementation amended the binding ability of mutant strains to spinach leaves.