Supporting Information

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SI Text

Strains, Plasmids and Growth Conditions. The *csgFcsgA* mutant was constructed using the Lambda red system to delete *csgA* (primers: csgA RED P1, csgA RED P2) in MHR592 (*csgF*) to create NDH58 (1). The resistance cassette was removed using flp-mediated excision. PCR was used to verify gene deletion. The pLR8 plasmid was created by amplifying *csgB* (primers: csgB F NcoI, pLR8 R PstI) from MC4100 and inserting it into the NcoI/PstI sites of pLR1. pAN23 was created by amplifying *csgF* without the predicted signal sequence (m*csgF*) (primers: mcsgF F NdeI, csgF R HindIII) from MC4100 and inserting it into the NdeI/HindIII sites of a pET28 vector, which adds an N-terminal His-tag and thrombin cleavage site (P. Ghosh laboratory, University of California at San Diego). pLR73 contains *csgF* inserted into the NcoI/PstI sites of pLR1; pLR75 contains *csgF* inserted into the SacI/PstI sites of pBAD33 (2). GenBank accession numbers for the sequences of these plasmid inserts are: pLR73, EU199782; and pLR75, EU199783. YESCA agar, per liter: 10g Casamino acids, 1g yeast extract, 20g agar; for CR plates, add 50 μg/ml Congo red.

Production of Polyclonal Serum Against His-mCsgF. For expression and purification of a his-tagged, mature form of CsgF (His-mCsgF), *E. coli* strain BL21(DE3) harboring pAN23 was grown in a 5L fermentor with 50 μg/ml kanamycin to OD_{600} 3.0 before induction with 0.15 mM IPTG for 1 h. His-mCsgF was recovered from the cytoplasmic fraction and affinity purified using Talon metal affinity resin (Clontech) and Q column resin (GE Healthcare). Affinity purified His-mCsgF was further concentrated (Millipore concentrators) and purified by SDS/PAGE; the major 15 kDa band was cut from the gel and sent to SigmaGenosys for rabbit immunization. The specificity for CsgF was confirmed by immunoblot analysis.

Immunoblot Analysis. For whole cell immunoblot, bacteria were scraped from YESCA plates, resuspended in PBS, and normalized by OD_{600}. A cell suspension volume corresponding to 1 optical density unit (ODU = 1 ml of OD_{600} = 1.0) was collected for each sample (i.e., whole cells + PBS, so that no protein was lost by pelleting of the bacteria and aspiration of PBS; these volumes were in the 15–30 μl range). For non-FA-treated samples, cell suspensions were brought to 200 μl using SDS loading buffer. For FA-treated samples, FA was added to cell suspensions to 70%, the acid evaporated in a vacuum centrifuge, the pellet resuspended in 200-μl SDS sample buffer, and pH adjusted with 1N NaOH, if necessary. For plug samples, a circular plug (d = 8 mm) was cut from the agar and collected. Plus/minus FA treatment of plugs was as above for whole cells, except that FA-treated plugs were solubilized in 100 μl of 96% FA. All samples were boiled for 5 min before SDS/PAGE in 15% acrylamide gels; resolved proteins were transferred to nitrocellulose membrane overnight at 4 °C at 12V in 25-mM CAPS, pH 11.2. Blocking: 4 °C overnight, rocking in 1× TBST, 1.5% milk, 1.5% BSA. Primary antibody: RT 1h at 1:5,000 dilution in blocking buffer (exception: CsgB anti-sera used at 1:2,000 dilution). Horseradish peroxidase conjugated secondary antibody (Pierce): RT 1 h at 1:10,000 dilution in blocking buffer. Detection: Supersignal West Femto chemiluminescent substrate (Pierce).

Immunofluorescence Microscopy. Intact cells were scraped from YESCA agar and normalized by OD_{600} as described above. Of each strain, 0.5 ODU was fixed in 5% formalin for 30 min, spun onto polylysine coated glass slides, washed in filter-sterile PBS, and blocked in PBST + 2% BSA for 45 min at RT. Slides were washed 3 times for 5 min in filter-sterile PBS then stained with a 1:5,000 dilution polyclonal rabbit α-CsgF serum (or PBS-negative control) for 1 h at RT; slides were washed 3 times for 5 min in filter-sterile PBS; secondary staining used a 1:1,000 dilution of FITC conjugated goat α-rabbit antibody (Molecular Probes), incubated for 1 h at RT; slides were washed 5 times for 5 min in filter-sterile PBS, the fourth wash included Hoescht dye. Cells were also probed with the α-CsgF antibody by a whole cell batch procedure before fixation with 5% formalin, with similar results. Slides were visualized on a Zeiss Axioskope Fluorescence microscope.

1. Datensko KA, Wanner BL (2000) One-step inactivation of chromosmal genes in Escherichia coli K-12 using PCR products. *Proc Natl Acad Sci USA* 97:6640–6645.
2. Guzman LM, Belin D, Carson MJ, Beckwith J (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol* 177:4121–4130.

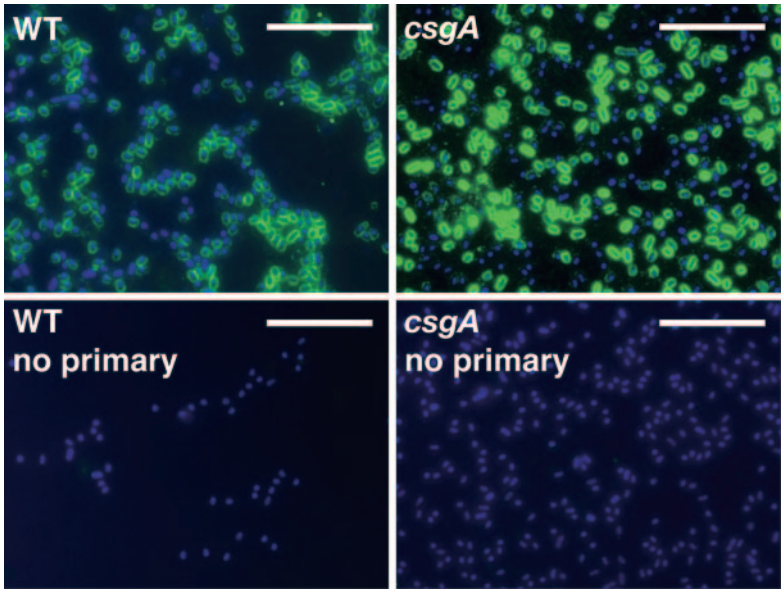

Fig. S1. CsgF localization. Whole cells of wild type (WT, MC4100) and *csgA* (LSR10) probed with rabbit polyclonal α-CsgF antibody or PBS (no primary), then FITC conjugated goat α-rabbit antibody. Each image is an overlay of the FITC channel (*green*, CsgF) and the DAPI channel (*blue*, DNA, Hoechst dye). (Scale bars: 10 μm.)

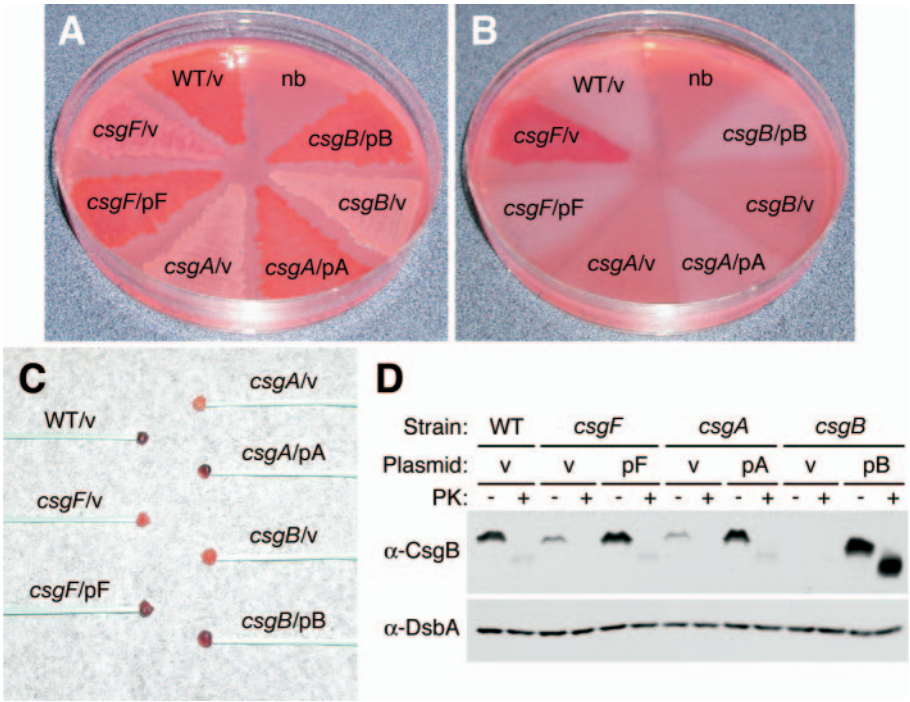

Fig. S2. Complementation of curli mutants; CsgB is extracellular in all strains in Fig. 3.(*A*–*C*) Congo red supplemented YESCA (CR-YESCA) agar plate containing wild type/v (MC4100/pLR1), *csgF*/v (MHR592/pLR1), *csgF*/pF (MHR592/pLR73), *csgA*/v (LSR10/pLR1), *csgA*/pA (LSR10/pLR5), *csgB*/v (MHR261/pLR1) and *csgB*/pB (MHR261/pLR8) after 48 h of growth (*A*) and the same plate after bacterial lawns were removed (*B*). One section of the plate contained no bacterial lawn (nb) as a color control. Bacterial cells (*C*) removed from the CR-YESCA plate in (*A*). (*D*) Whole cells of the same strains in (*A*–*C*) were collected after 48 h of growth on YESCA agar and treated with 0 or 100 μg/ml of proteinase K (PK), and then analyzed by α-CsgB immunoblot. An α-DsbA immunoblot was used as a positive control for cell integrity. v, empty matched vector.

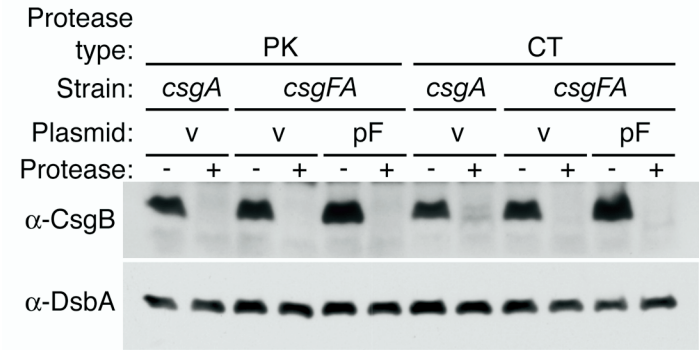

Fig. S3. CsgB is extracellular in all strains in Fig. 5. Whole cells of *csgA*/v (LSR10/pLR1), *csgFcsgA*/v (NDH58/pLR1), and *csgFcsgA*/pF (NDH58/pLR73) were collected after 48 h of growth on YESCA agar. Whole cells were treated with 0 or 10 μg/ml of proteinase K (PK) or chymotrypsin (CT), and then analyzed by α-CsgB immunoblot. An α-DsbA immunoblot was used as a positive control for cell integrity. v, empty matched vector.

Table S1. Strains and plasmids

Strain/ plasmid	Common name	Relevant characteristics	Reference
MC4100	wild type	F_ *araD139* Δ(*argF-lac*)*U169 rspL150*(*strR*) *relA1 flbB5301 deoC1 ptsF25 rbsR*	(1)
MHR261	*csgB*	MC4100 *csgB*	(2)
MHR480	*csgE*	MC4100 *csgE*	(3)
MHR592	*csgF*	MC4100 *csgF*	(3)
LSR1	*csgG*	MC4100 *csgG*::Tn*105*	(4)
LSR10	*csgA*	MC4100 *csgA*	(3)
NDH58	*csgFcsgA*	MHR592 *csgA*	Present study
BL21(DE3)		Protein expression strain	Stratagene
C600	C600	F- *thr leu thi lac tonA*	(5)
pLR1	vector, v	*csgBA* promoter in pACYC177	(4)
pLR5	p*csgA*, pA	*csgA* in pLR1	(6)
pLR8	p*csgB*, pB	*csgB* in pLR1	(7)
pLR73	p*csgF*, pF	*csgF* in pLR1	Present study
pLR75	p*csgF*+, pF+	*csgF* in the SacI/PstI sites of pBAD33a, an arabinsose-inducible expression vector	Present study
pTrc99a	vector+, v+	IPTG-inducible expression vector	Pharmacia Biotech
pMC1	p*csgG*+, pG+	*csgG* in pTrc99a	(3)
pAN23		m*csgF* in pET28	Present study

References for the Strain/plasmid list:

1. Casadaban MJ (1976) Transposition and fusion of the lac genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J Mol Biol* 104:541–555.
2. Hammar M, Arnqvist A, Bian Z, Olsen A, Normark S (1995) Expression of two csg operons is required for production of fibronectin- and congo red-binding curli polymers in *Escherichia coli* K-12. *Mol Microbiol* 18:661–670.
3. Chapman MR*, et al.* (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science* 295:851–855.
4. Robinson LS, Ashman EM, Hultgren SJ, Chapman MR (2006) Secretion of curli fibre subunits is mediated by the outer membrane-localized CsgG protein. *Mol Microbiol* 59:870–881.
5. Campbell A (1961) Sensitive mutants of bacteriophage lambda. *Virology* 14(1):22–32.
6. Wang X, Hammer ND, Chapman MR (2008) The molecular basis of functional bacterial amyloid polymerization and nucleation. *J Biol Chem* 283:21530–21539.
7. Hammer ND, Schmidt JC, Chapman MR (2007) The curli nucleator protein, CsgB, contains an amyloidogenic domain that directs CsgA polymerization. *Proc Natl Acad Sci U S A* 104:12494–12499.

Table S2. Primer sequences used to make knock-out strains and plasmids

Primer /gene	Sequence (5′→3′)
csgA RED P1	CCATTCGACTTTTAAATCAATCCGATGGGGGTTTTACATGAAACTTGTGTGTAGGCTGGGCTGCTTC
csgA RED P2	GGGCTTGCGCCCTGTTTCTGTAATACAAATGATGTATTAGTACTGCATATGAATATCCTCCTTAG
csgB F NcoI	gtttccATGgcgAAAAACAAATTGTTATTTATGATG
pLR8 R PstI	gtttctgcagTTAACGCTGTGTCACGCGAATAGCCATTTGC
mcsgF F NdeI	GGAATTCCATATGGGAACCATGACTTTCCAGTTCCGT
csgF R HindIII	CGCCAAGCTTTTAAAAATCGGTTGACTTATTTTGTAAACC