

Supplemental Figures and Tables

Figure S1. A CsgA homolog from *S. oneidensis* MR-1 (CsgA_{SO}) formed amyloid fibers *in vitro*. **A)** TEM of fibers formed by freshly purified CsgA_{SO} after incubated at room temperature for 24 h. The scale bar equals 500 nm. **B)** Circular dichroism (CD) analysis of CsgA_{SO} fibers.

Figure S2. Fibers formed by CsgA homologs, CsgB homologs or fibers formed by CsgA_{EC} in the presence of various seeds had similar stability. CsgA^{slowgo} was more sensitive to HFIP treatment. 22 μg fibers as indicated were treated with no HFIP or with 50% (v/v), 70%, 80%, 90% or 100% Samples were immediately dried on a SpeedVac. Samples were boiled in 2x SDS-loading dye for 5 min and loaded on 15% SDS-PAGE gels. Monomers dissociated from fibers by HFIP treatment migrated into the gel at 17 kDa.

Figure S3. Curli subunits didn't cross-seed with Aβ₁₋₄₂ or Sup35-NM. **A)** ThT fluorescence monitoring the polymerization kinetics of 40 μM freshly purified CsgA_{EC} alone (●), in the presence of 8% (w/w) Aβ₁₋₄₂ seeds (○) or 4% CsgA_{EC} seeds (Δ). **B)** Polymerization kinetics of 25 μM freshly purified Aβ₁₋₄₂ alone (●), in the presence of 8% Aβ₁₋₄₂ seeds (○) or 8% CsgA_{EC} seeds (Δ). **C)** 10 μM monomeric Sup35 NM polymerized on its own (●), or in the presence of 5% weak Sup35 NM seeds (▲), strong Sup35 NM seeds (◆), CsgA_{EC} seeds (□) or CsgB_{EC} seeds (Δ). **D)** 10 μM freshly purified CsgA polymerized with no seeds (●), with 5% weak Sup35 NM seeds (□) or 5% strong Sup35 NM seeds (Δ).

Figure S4. Aβ₁₋₄₂ didn't bind curli fibers efficiently. Sensorgrams of interactions between over 1 μM freshly prepared Aβ₁₋₄₂ monomers and CsgA_{EC} seeds **(A)** or CsgB_{EC} seeds **(B)**.

Figure S5. *E. coli csgB-* was complemented by CsgB homologs from *S. typhimurium* (pCsgB_{ST}) or *C. koseri* (pCsgB_{CK}). **A)** YESCA-CR agar plate with *E. coli csgB-* harboring an empty vector, pCsgB_{EC},

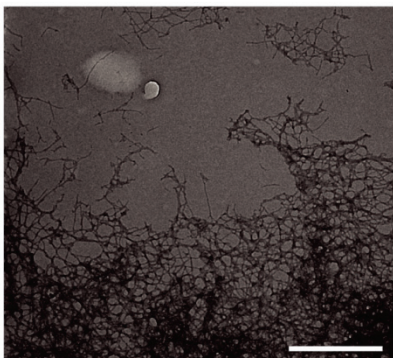
pCsgB_{ST} or pCsgB_{CK} after 48 hr incubation at 26°C. **B)** Western analysis of the whole cell lysates and bacteria with underlying agar of *E. coli csgB*- harboring the empty vector, pCsgB_{EC}, pCsgB_{ST} or pCsgB_{CK} after grown on YESCA agar at 26°C for 48 hr. Samples were pre-treated with (+) or without (-) HFIP before electrophoresis. **C)** TEM of *E. coli csgB*- mutant complemented with pCsgB_{EC}, pCsgB_{ST} or pCsgB_{CK} after grown on YESCA agar at 26°C for 48 hr. Scale bars equal to 500 nm.

Figure S6. *S. typhimurium csgB*- (A^+B^-) outgrew *E. coli csgA*- (A^-B^+) in the mixed colony. **A)** The percentage of *S. typhimurium* or *E. coli* curli mutants in the mixed colony. Overnight cultures of *E. coli* and *S. typhimurium* mutants were normalized by OD₆₀₀, mixed at 1:1 (v/v) ratio and spotted on YESCA plates. After 3 days, mixed colonies were suspended in PBS, series diluted and plated on YESCA-CR plates. *S. typhimurium* curli mutants formed pink colonies on YESCA-CR agar while *E. coli* curli mutants formed white colonies. Colony forming units (CFU) of pink or white colonies were measured and plotted as 100% stacked columns. **B)** Western analysis bacteria with underlying agar of *E. coli* A^-B^+ /*S. typhimurium* A^+B^- mixed colonies or *E. coli* A^+B^- /*S. typhimurium* A^-B^+ mixed colonies. Samples were pre-treated with (+) or without (-) HFIP before electrophoresis and CsgA was probed by α CsgA antibody.

Figure S7. The increase in bacterial adherence was CsgB-dependent. **A)** Overnight culture of *E. coli*, *S. typhimurium* or 1:1 (OD₆₀₀ ratio) mixture of both species as indicated in the figure were spread on YESCA and incubated at 26°C for 3 d. Bacteria were washed in 1ml PBS with vigorous shaking for 30 min. OD₆₀₀ of non-adhesive bacteria (both *E. coli* and *S. typhimurium*) and bacteria adhere to the agar after washing were measured as described in the Experimental Procedures. The percentage of adhered bacteria in total population was graphed **B)** *E. coli*, *S. typhimurium* or the mixed culture grown on YESCA agar for 3 d were washed in PBS with vigorous shaking. Bacteria washed off into PBS or adhere to the agar were series diluted and spread on YESCA-CR plates. *E. coli* curli mutants stained white and *S. typhimurium* curli mutant stained pink on YESCA-CR agar. Percentage of adhesive *S. typhimurium* was determined by CFU of pink colonies.

Figure S1

A



B

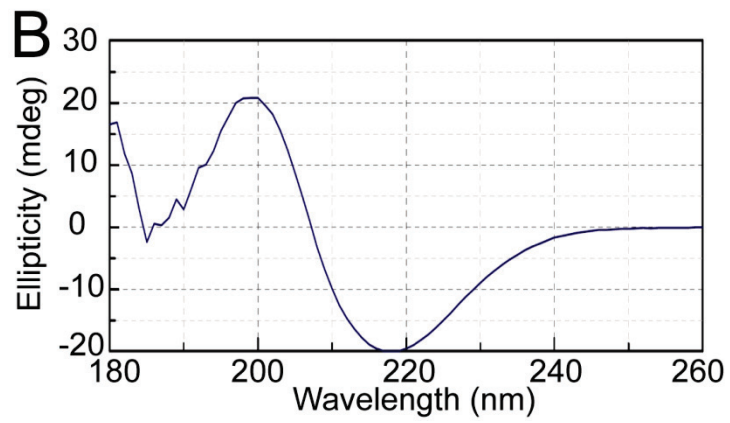


Figure S2

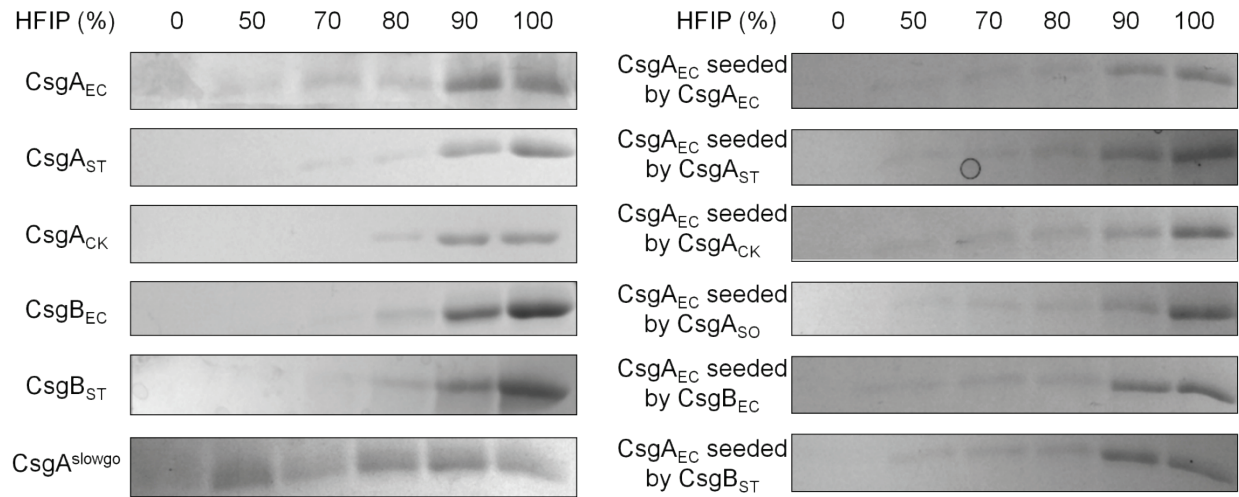


Figure S3

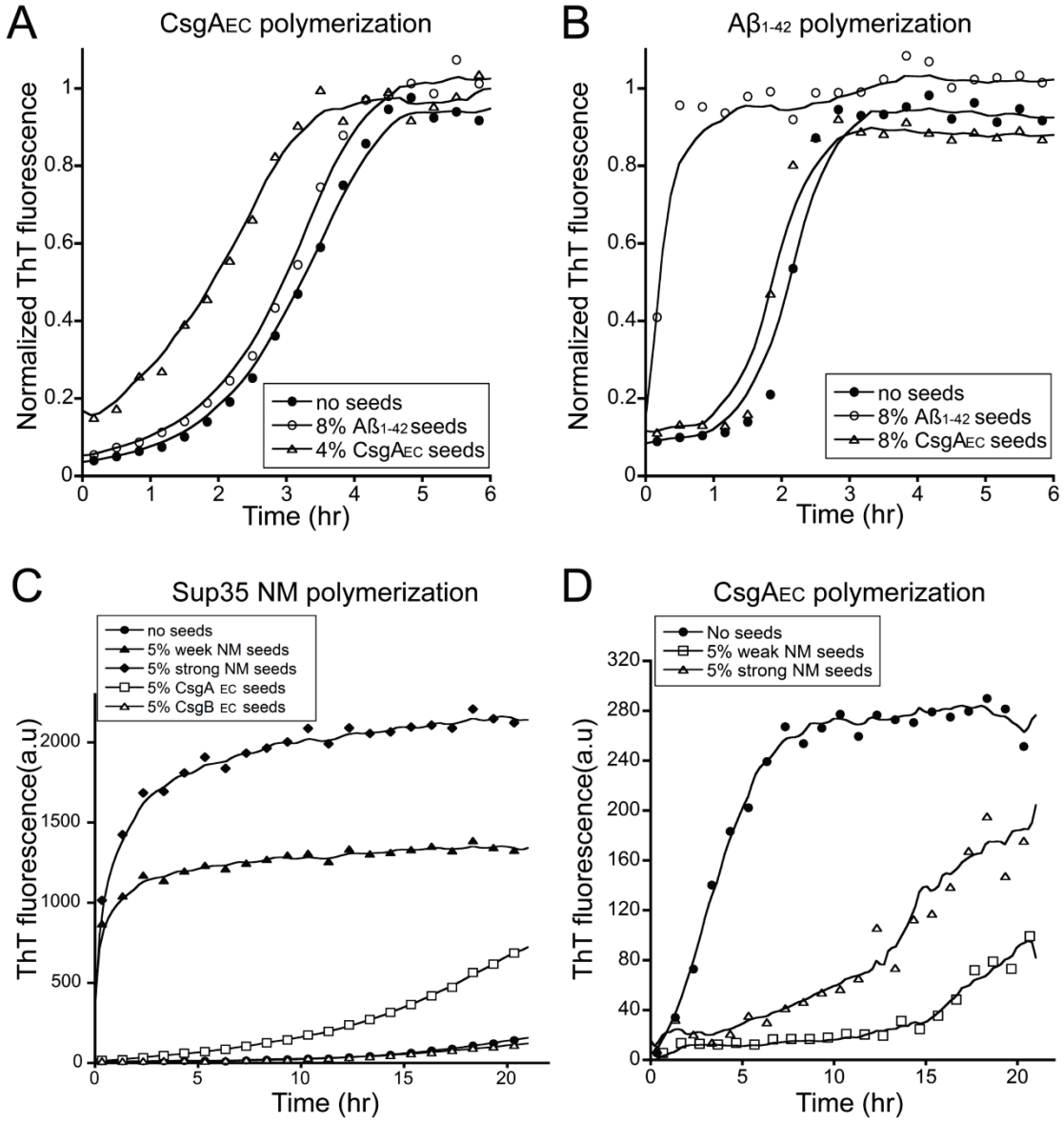


Figure S4

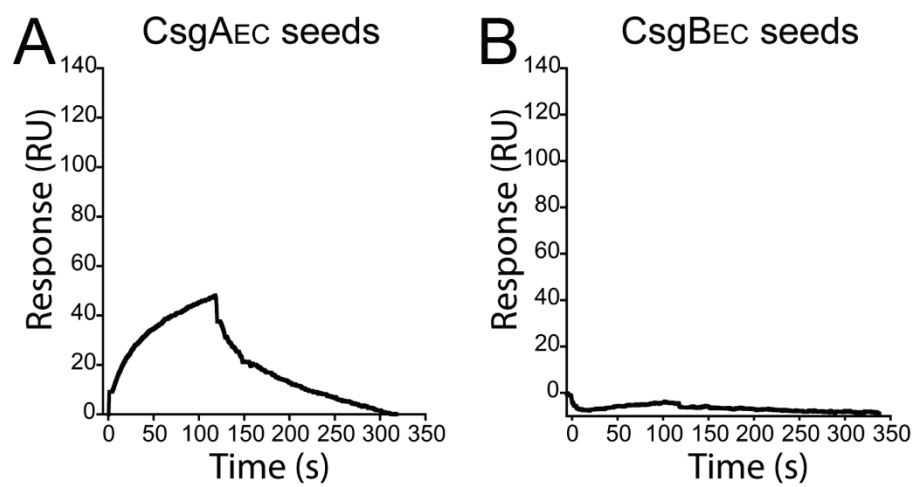


Figure S5

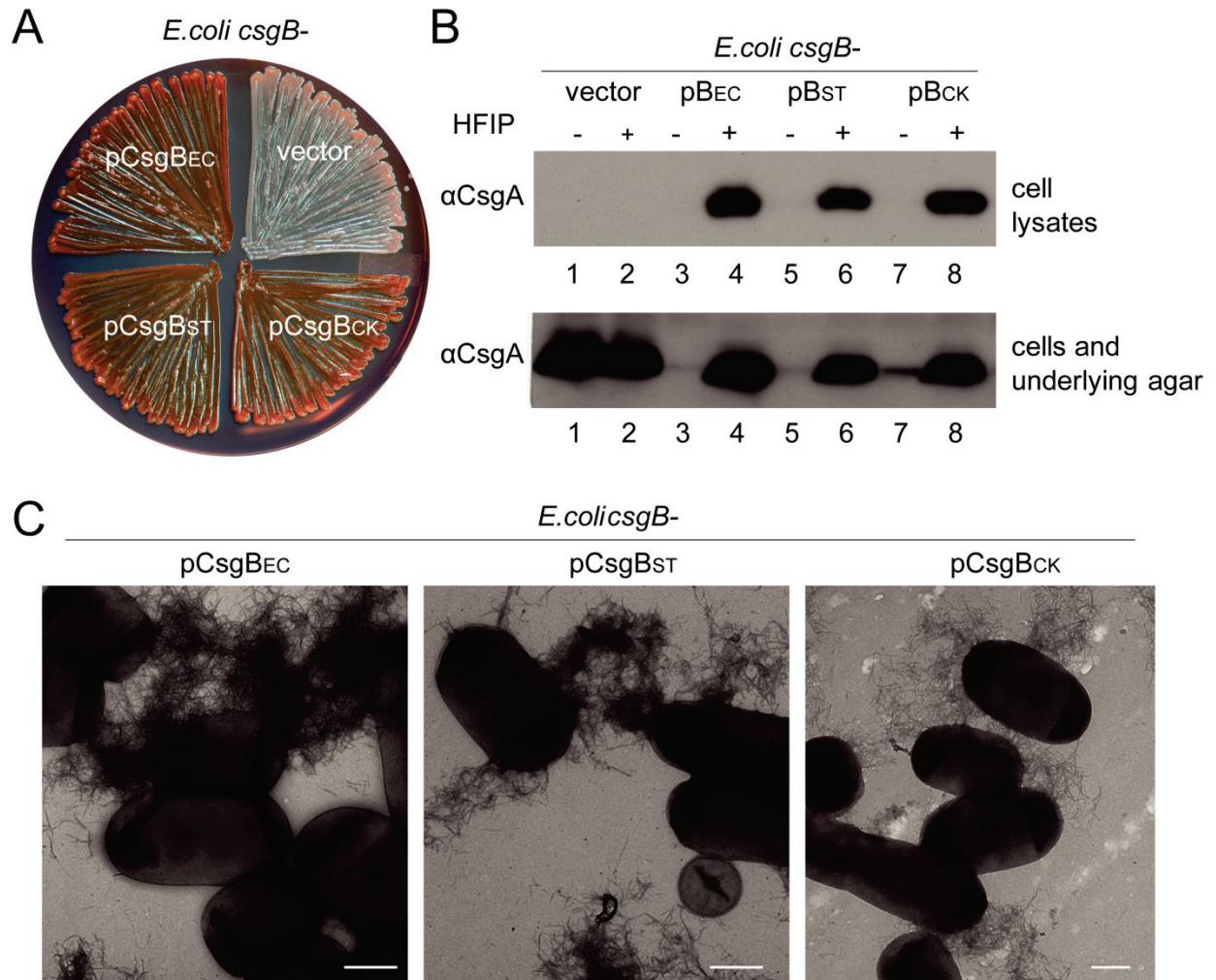
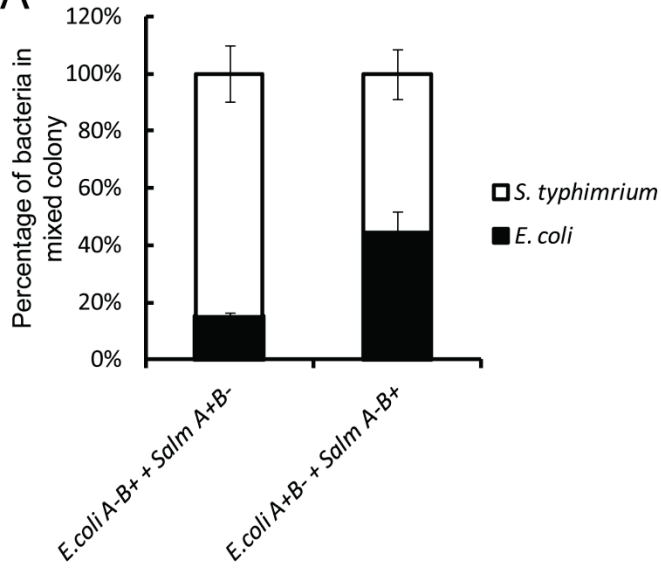


Figure S6

A



B

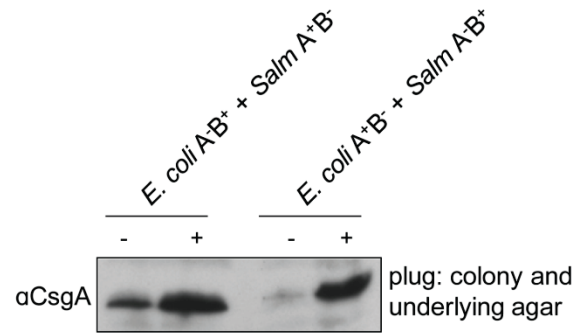
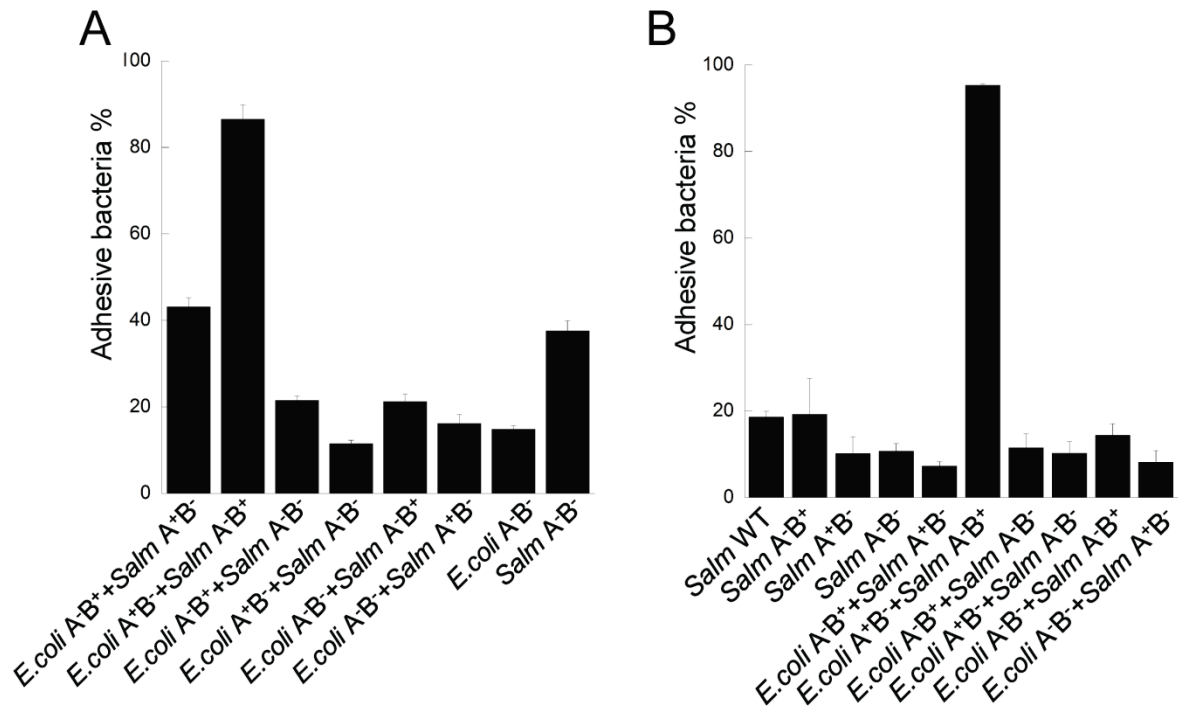


Figure S7



Supplemental Table 1. Strains used in this study

Strains	Relevant characteristics	References
MC4100	F- <i>araD139</i> Δ (<i>argF-lac</i>) U169 <i>rpsL150(strR)</i> <i>relA1 fibB5301 deoC1 ptsF25 rbsB</i>	(1)
<i>E. coli csgA-</i> (LSR10)	MC4100 Δ <i>csgA</i>	(2)
<i>E. coli csgB-</i> (MHR261)	MC4100 Δ <i>csgB</i>	(3)
<i>E. coli csgBA-</i> (LSR13)	MC4100 Δ <i>csgBA</i>	(4)
<i>S. typhimurium</i> LT2	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium str. LT2	From Dr. Blaise Boles
<i>S. typhimurium csgA-</i>	<i>S. typhimurium</i> LT2 <i>csgA::kan</i>	This study
<i>S. typhimurium csgB-</i>	<i>S. typhimurium</i> LT2 <i>csgB::kan</i>	This study
<i>S. typhimurium csgBA-</i>	<i>S. typhimurium</i> LT2 <i>csgBA::kan</i>	This study
NEB 3016	<i>fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11</i> <i>R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-</i> <i>210::Tn10--Tet^S) endA1 Δ(mcrC-</i> <i>mrr)114::IS10 [mini-lacI^F (Cam^R)]</i>	NEB

Supplemental Table 2. Plasmids used in this study

Plasmids	Relevant characteristics	References
pLR2	Vector with <i>csgBA</i> promoter	(5)
pCsgA _{EC}	<i>E. coli csgA</i> cloned into pLR2. Previously named as pLR5.	(4)
pCsgA _{ST}	<i>S. typhimurium csgA</i> cloned into pLR2.	This study
pCsgA _{CK}	<i>C. koseri csgA</i> cloned into pLR2.	This study
pCsgA _{SO}	<i>S. oneidensis csgA</i> cloned into pLR2	This study
pCsgBA _{EC}	<i>E. coli csgBA</i> cloned into pLR2, previously named as pLR30.	This study
pCsgB _{EC}	<i>E. coli csgB</i> cloned into pLR2. Previously named as pLR8.	(6)
pCsgB _{ST}	<i>S. typhimurium csgB</i> cloned into pLR2.	This study
pCsgB _{CK}	<i>C. koseri csgB</i> cloned into pLR2.	This study
pCsgB _{SO}	<i>S. oneidensis csgB</i> cloned into pLR2.	This study
pET11d	Expression vector	NEB
pNH11	C-terminal His ₆ tagged <i>E. coli csgA</i> cloned into pET11d	(7)
pET11d-CsgA _{ST}	C-terminal His ₆ tagged <i>S. typhimurium csgA</i> cloned into pET11d	This study
pET11d-CsgA _{CK}	C-terminal His ₆ tagged <i>C. koseri csgA</i> cloned into pET11d	This study
pET11d-CsgA _{SO}	C-terminal His ₆ tagged <i>S. oneidensis csgA</i> cloned into pET11d	This study
pAN1	C-terminal His ₆ tagged <i>E. coli csgB</i> cloned into pET11d	(7)
pET11d-CsgB _{ST}	C-terminal His ₆ tagged <i>S. typhimurium csgB</i> cloned into pET11d	This study
pAH9	Plasmid for mCherry expression	(8)
pAH16	Plasmid for YFP expression	(8)

Supplemental Table 3. Primers used in this study

Primer	Primer Sequence	Constructs
WD1	5'-CACGACCCATGGCGAAACTTTTAAAAGTGGCAG-3'	pCsgA _{ST} , pCsgA _{CK}
WD2	5'-CAGCTTGGATCCTTAATACTGGTTAGCCGTGG-3'	pCsgA _{ST} , pCsgA _{CK}
YZ15-f	5'-GCGTTTCCATGGGCGTCGTTCCACAATGGGGCGG-3'	pET11d-CsgA _{ST}
YZ15-r	5'-GTTTAAAGCTTGGATCCTTAGTGATGGTGGTGGT GATACTGGTTAGCCGTGGCGTTG-3'	pET11d-CsgA _{ST} pET11d-CsgA _{CK}
YZ16-f	5'-GCGTTTCCATGGGTGTTGTTCCGCAGTGGGGCGGT-3'	pET11d-CsgA _{CK}
YZ17-f	5'-GCGCCATGGCGAAAAACAAATTGTTATTTATG-3'	pCsgB _{ST} , pCsgB _{CK}
YZ21-r	5'-GCGGGATCCTTAACGTTGCGTAACGCG-3'	pCsgB _{CK}
YZ22-r	5'-GCGGATCCTTAGCGTTGGGTGACGCGAATAG-3'	pCsgB _{ST}
YZ22-f	5'-GCTACCATGGCGACAAATTATGATCTG-3'	pET11d-CsgB _{ST}
YZ22-r	5'-GCGGATCCTTAGTGATGGTATGGTGGTGGCGTTGGGT GACGCGAATAG-3'	pET11d-CsgB _{ST}
YZ25-f	5'-GCGTTTCCATGGCAAGTACGATCAACGAAATC	pET11d-CsgA _{SO}
YZ25-r	5'-GCGGATCCTTAGTGATGGTGGTGGTGGTATTGCA CTACAGTCG-3'	pET11d-CsgA _{SO}
YZ47	5'-CAACGCTAATACCGTTACGACTTTTAAATCAATCCGAT GGGGGTTTTACCCACCAAACACCCCCAAAACC-3'	<i>S. typhimurium</i> csgA-
YZ48	5'-CAGGGCTTATGCCCTGTTTTTTTTATTAGCGCAGACGCT AAACACACAACCACACCACACCAC-3'	<i>S. typhimurium</i> csgA- <i>S. typhimurium</i> csgBA-
YZ50	5'-CAAGGTAATAGATAATTTTCGCTATGTACGACCAGGTC CAGGGTGACAGCCACCAAACACCCCCAAAACC-3'	<i>S. typhimurium</i> csgB- <i>S. typhimurium</i> csgBA-
YZ51	5'-AAGTTTCATGGTAAAACCCCATCGGATTGATTTAAAA GTCGTAACGGTACACACAACCACACCACACCAC-3'	<i>S. typhimurium</i> csgB-

Reference

1. Goldberg, R. B., Bender, R. A., and Streicher, S. L. (1974) Direct selection for P1-sensitive mutants of enteric bacteria. *J Bacteriol* **118**, 810-814
2. Chapman, M. R., Robinson, L. S., Pinkner, J. S., Roth, R., Heuser, J., Hammar, M., Normark, S., and Hultgren, S. J. (2002) Role of Escherichia coli curli operons in directing amyloid fiber formation. *Science* **295**, 851-855
3. Hammar, M., Bian, Z., and Normark, S. (1996) Nucleator-dependent intercellular assembly of adhesive curli organelles in Escherichia coli. *Proc Natl Acad Sci U S A* **93**, 6562-6566
4. Wang, X., Hammer, N. D., and Chapman, M. R. (2008) The molecular basis of functional bacterial amyloid polymerization and nucleation. *J Biol Chem* **283**, 21530-21539
5. Robinson, L. S., Ashman, E. M., Hultgren, S. J., and Chapman, M. R. (2006) Secretion of curli fibre subunits is mediated by the outer membrane-localized CsgG protein. *Mol Microbiol* **59**, 870-881
6. Hammer, N. D., Schmidt, J. C., and Chapman, M. R. (2007) The curli nucleator protein, CsgB, contains an amyloidogenic domain that directs CsgA polymerization. *Proc Natl Acad Sci U S A* **104**, 12494-12499
7. Hammer, N. D., McGuffie, B. A., Zhou, Y., Badtke, M. P., Reinke, A. A., Brännström, K., E., G. J., Olofsson, A., Almqvist, F., and Chapman, M. R. (Submitted to J Mol Biol) The C-terminal repeating units of CsgB direct bacterial functional amyloid nucleation.
8. Malone, C. L., Boles, B. R., Lauderdale, K. J., Thoendel, M., Kavanaugh, J. S., and Horswill, A. R. (2009) Fluorescent reporters for Staphylococcus aureus. *J Microbiol Methods* **77**, 251-260