Supplemental figures and tables

Supplemental Fig. 1. Negative-stain EM micrographs of CsgA, ΔR2, ΔR3 and ΔR4 fibers assembled *in vivo* at high magnification.

Negative-stain EM micrographs of wild-type strain MC4100 and *csgA* mutant cells containing the indicated plasmids. Cells were grown on YESCA plates for 48 hrs at 26°C prior to staining with uranyl acetate. Scale bars are equal to 200 nm.

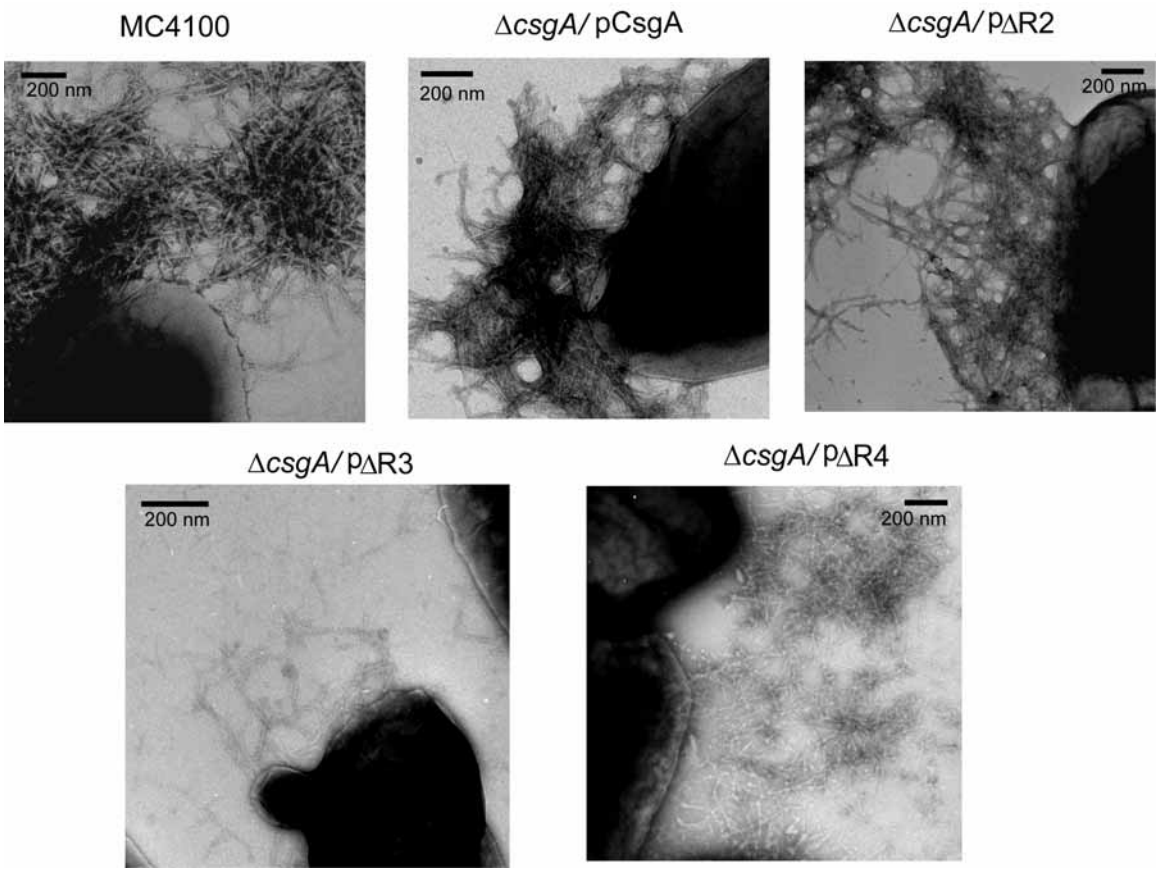
Supplemental Fig. 2. Negative-stain EM micrographs of *csgA* cells expressing CsgA, ΔR1 or ΔR5 for a long-term growth.

Negative-stain EM micrographs of *csgA* mutant cells containing the indicated plasmids. Cells were grown on YESCA plates for 100 hrs at 26°C prior to staining with uranyl acetate. Scale bars are equal to 500 nm.

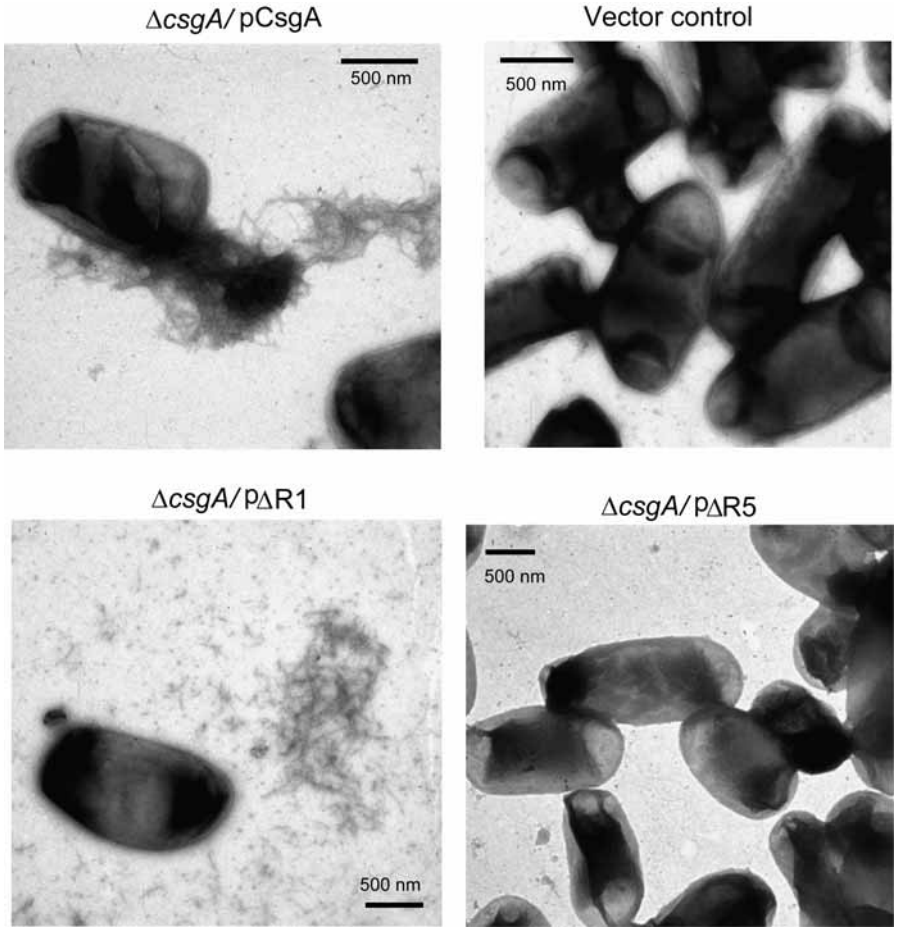
Supplemental Fig. 3. *In vitro* polymerization of CsgA mutant proteins under the quiescent condition.

13 μM CsgA (A) and ΔR5 at various concentrations (B) were incubated at room temperature without agitation in the presence of 0.02% NaN_3. At the indicated time points, samples were withdrawn, ThT was added at a concentration of 20 μM and fluorescence was measured at 495 nm after excitation at 438 nm by a Spectramax M2 plate reader (Molecular Devices, Sunnyvale, CA). ThT fluorescence was normalized as described in the Materials and Methods.

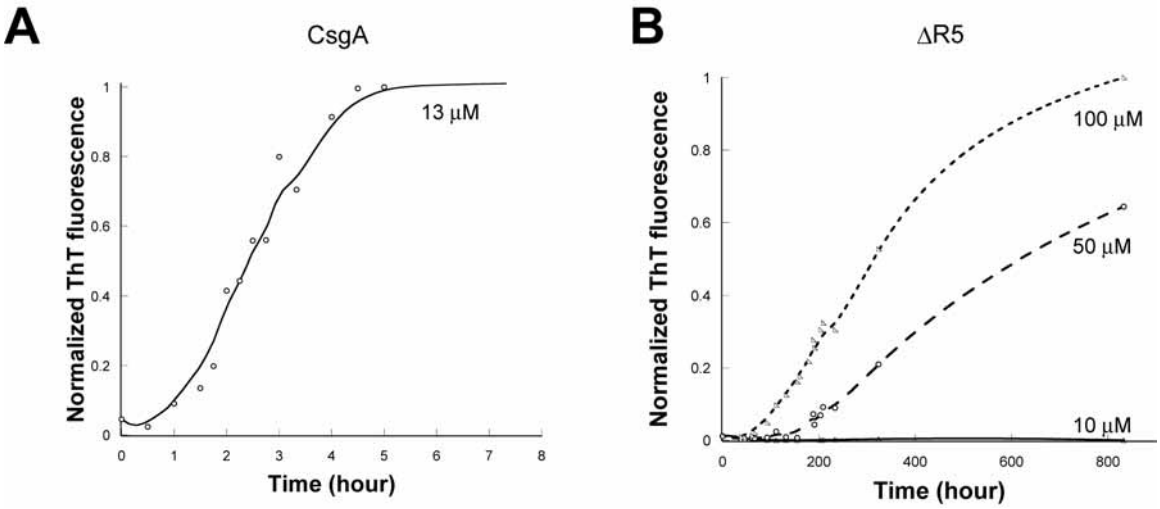
Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Table 1. Strains and plasmids used in this study[a]

Strains or Plasmids	Relevant characteristics	References	Primer used
Strains			
csgA (LSR10)	MC4100 Δ*csgA*	(Chapman et al., 2002)	
csgBA (LSR13)	MC4100 Δ*csgBA*	(Hammer et al., 2007)	
LSR12	C600 Δ*csgBA and*Δ*csgDEFG*	(Chapman et al., 2002)	
Plasmids			
pCsgA (pLR5)	*csgA* sequence in pLR2	Hultgren lab	
pLR2	Control vector containing csg*BA* promoter	(Robinson et al., 2006)	
pΔR1	*csgA* without R1(S^{43} to N^{65}) in pLR2	This Study	FpLR5, RpLR5, pΔR1 P1, P2
pΔR2	*csgA* without R2 (S^{66} to D^{87}) in pLR2	This study	FpLR5, RpLR5, pΔR2 P1, P2
pΔR3	*csgA* without R3 (S^{88} to N^{110}) in pLR2	This study	FpLR5, RpLR5, pΔR3 P1, P2
pΔR4	*csgA* without R4 (S^{111} to N^{132}) in pLR2	This study	FpLR5, RpLR5, pΔR4 P1, P2
pΔR5	*csgA* without R5 (S^{133} to Y^{151}) in pLR2	This study	FpLR5, pΔR5 P1
pΔR1&5	*csgA* without R1 and R5 in pLR2	This study	FpLR5, pΔR5 P1[b]

[a] Vectors used for expression and purification are not listed. These vectors were constructed by insertion of PCR amplified mutant *csgA* sequences with C-terminal hexahistidine tag into NdeI/EcoR1 sites in pMC3 (Chapman et al., 2002) replacing sequence encoding CsgA-his.

[b] pΔR1 was used as template for PCR to make pΔR1&5.

Supplemental Table 2. Sequence of primers used in this study

Primer Name	Sequence
FpLR5[a]	5' CATGCCATGGCGAAACTTTTAAAAGTAGC 3'
RpLR5[b]	5' CGGGATCCTGTATTAGTACTGAT 3'
pΔR1 P1[c]	5' AATAGTCAAGTCAGAATTTGGGCCGCTATT 3'
pΔR1 P2[d]	5' AATAGCGGCCCAAATTCTGACTTGACTATT 3'
pΔR2 P1	5' GATCGATTGAGCTGTTACGGGCATCA 3'
pΔR2 P2	5' TGATGCCCGTAACAGCTCAATCGATC 3'
pΔR3 P1	5' CCGTCATTTCAGAGTCATCTGAGCCCT 3'
pΔR3 P2	5' AGGGCTCAGATGACTCTGAAATGACGG 3'
pΔR4 P1	5' CGTTGACGGAGGAATTTTTGCCGTTC 3'
pΔR4 P2	5' GAACGGCAAAAATTCCTCCGTCAACG 3'
pΔR5 P1	5' CGGGATCCTGTATTAGTTAGATGCAG 3'

[a] FpLR5 is paired to noncoding strand immediately upstream of the start codon of *csgA* in pLR5.

[b] RpLR5 is paired to coding strand immediately downstream of the stop codon of *csgA* in pLR5.

[C] The primers with odd number such as pΔR1 P1 are paired to the coding strand of *csgA* template.

[d] The primers with even number such as pΔR1 P2 are paired to the noncoding strand of *csgA* template.

Supplemental References:

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.

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. **93**, 6562-6566

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**, 870-88