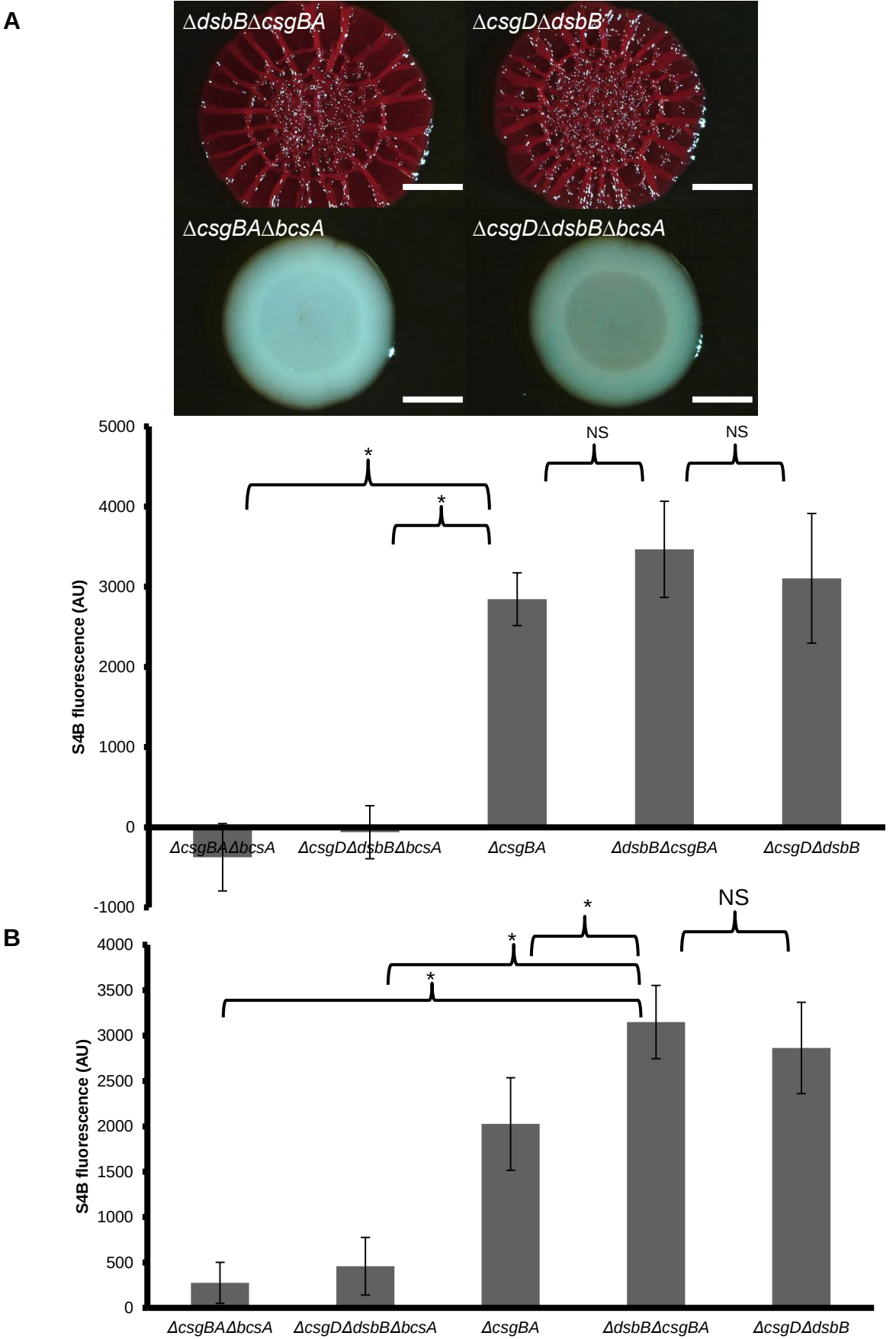
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ΔcsgDΔdsbB
ΔcsgBAΔbcsA
ΔcsgDΔdsbBΔbcsA
S4B fluorescence (AU)
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4000
3000
2000
1000
0
-1000
*
*
NS
NS
ΔcsgBAΔbcsA
ΔcsgDΔdsbBΔbcsA
ΔcsgBA
ΔdsbBΔcsgBA
ΔcsgDΔdsbB
B
S4B fluorescence (AU)
4000
3500
3000
2500
2000
1500
1000
500
0
*
*
*
NS
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ΔcsgDΔdsbBΔbcsA
ΔcsgBA
ΔdsbBΔcsgBA
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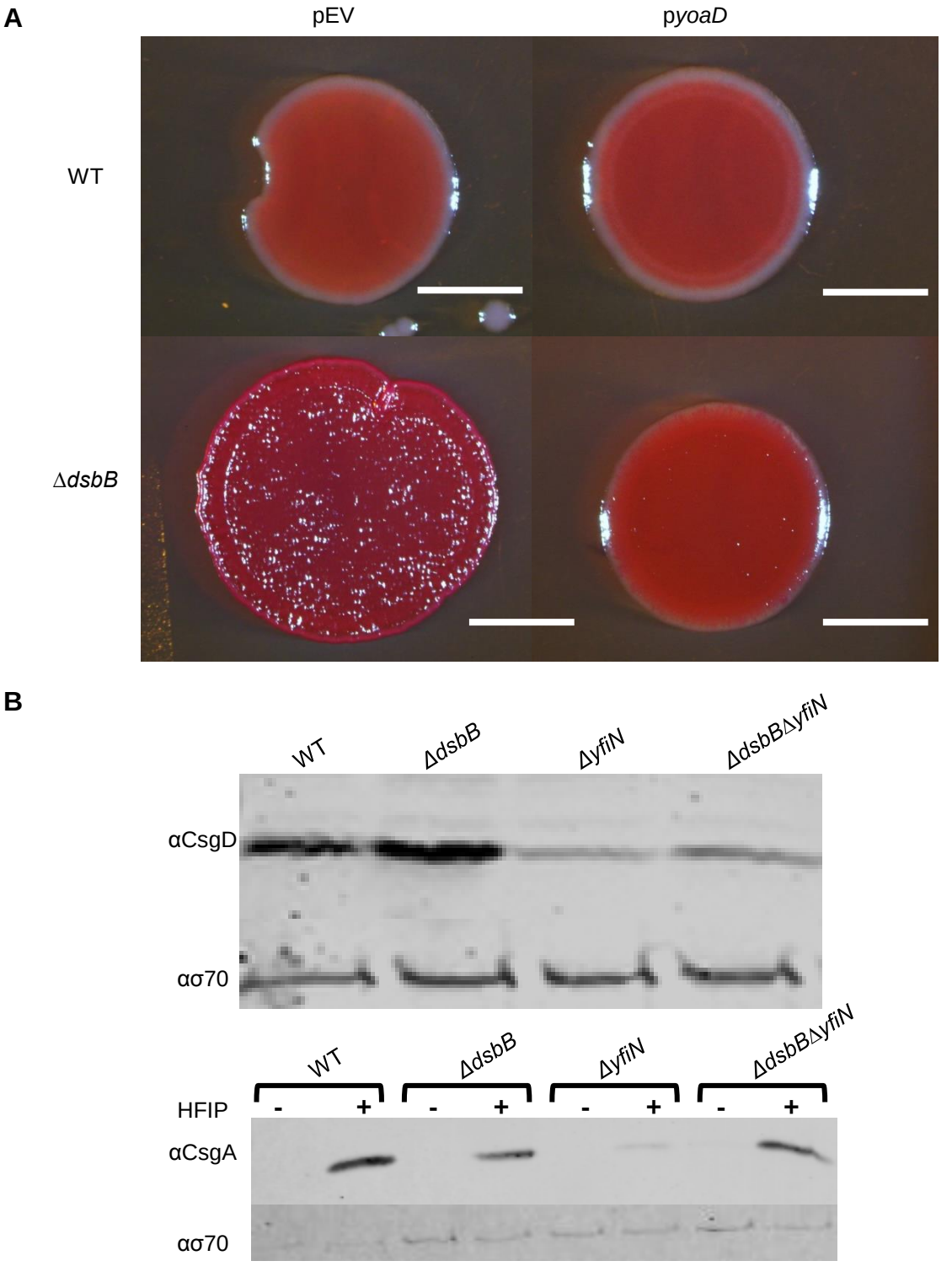
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pyoaD
WT
ΔdsbB
B
WT
ΔdsbB
ΔyfiN
ΔdsbBΔyfiN
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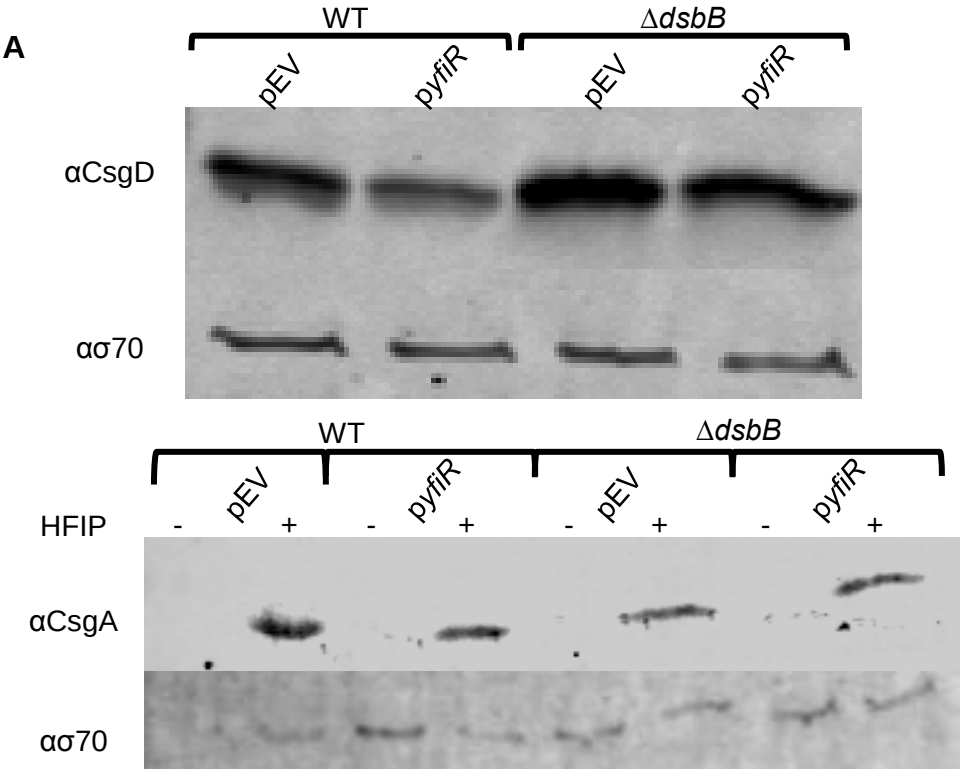
HFIP
- + - + - + - +
αCsgA
ασ70


A
WT
ΔdsbB
pEV
pyfiR
pEV
pyfiR
αCsgD
ασ70
WT
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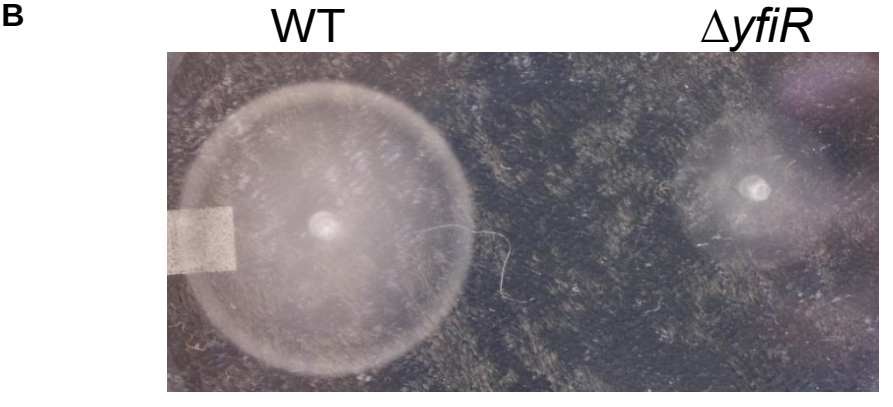
pyfiR
+
αCsgA
ασ70


B
WT
ΔyfiR

Supplemental Figure 1. UTI89 WT, Δ*dsbB,* and Δ*csgD*Δ*dsbB* have increased binding to the cellulose stain S4B even on LB plates. A) In the top images, colonies were grown for 48 hours at 26°C on YESCA CR. Scale bars are 0.25cm. In the graph, the colonies were grown on YESCA for 48 hours at 26°C were stained with Pontamine Fast Scarlet 4B (S4B). Fluorescence readings were normalized by OD_{600} and unstained cells fluorescence were subtracted from the stained cells fluorescence. Error bars are the standard deviation from biological triplicate samples. P-values were calculated using the student's two-tailed t-test (*P-value<0.04). B) Colonies grown on LB plates at 26°C for 48 hours were stained with S4B. Fluorescence readings were normalized by OD_{600} and unstained cells fluorescence were subtracted from the stained cells fluorescence. Error bars are the standard deviation from biological triplicate samples. P-values were calculated using the student's two-tailed t-test (P-value<0.03).

Supplemental Figure 2. Δ*dsb* phenotype is dependent on c-di-GMP, and YfiN is necessary for normal CsgD expression. A) WT and Δ*dsbB* were transformed with pCKR101-EV and pCKR101-*yoaD.* Colonies were grown on YESCA CR plates with 100μM IPTG at 26°C for 24 hours. B) Colonies were grown at 26°C for 48 hours for αCsgA and 24 hours for αCsgD Western blots.

Supplemental Figure 3. *yfiR* overexpression decreases CsgD levels in WT and Δ*dsbB* colonies. A) Western Blot analysis was performed on colonies grown at 26°C on YESCA plates. Colonies used to analyze CsgA protein levels were grown for 48 hours, while Western Blots used to analyze CsgD levels were grown for 24 hours. CsgA colony preps were treated with HFIP in order to solubilize CsgA fibers. ασ70 blots were

used as loading controls. B) 1 µL of culture was inoculated into .25% YESCA motility agar. Plates were incubated at 37°C for 5 hours.

Supplemental Table 1

Gene	Number	Encoded protein's function
bcsA	4	Cellulose synthase (3)
bcsB	8	Involved in biosynthesis of cellulose (3)
bcsC	11	Involved in biosynthesis of cellulose (3)
bcsE	2	Involved in biosynthesis of cellulose (3)
galU	3	UTP-glucose-1-phosphate uridylyltransferase, which produces UDP-D-glucose, the building block of cellulose (40)
nhaA	1	Sodium antiporter (41)
nrdB	1	B2 protein of ribonucleoside-diphosphate reductase. Involved in production of deoxynucleotides from nucleotides (42)
seqA	1	Involved in the regulation of DNA replication (44)
yfiN	1	Inner membrane diguanylate cyclase (46, 47)

Plasmid list

Plasmids	Reference	Notes
pRJ800	DePas *et al.* 2013	
pRJ800-*adrA*	DePas *et al.* 2013	
pRJ800-16s	DePas *et al.* 2013	
pCKR101	DePas *et al.* 2013(20)	
pCKR101-*yfiR*	This study	*yfIR* amplified with primers DH165 and DH166 subcloned into pCKR101 at the KpnI and XbaI sites.
pCKR101-*yfiR*^{His}	This study	*yfIR* amplified with primers DH165 and DH167 subcloned into pCKR101 at the KpnI and XbaI sites.
pCKR101-*yoaD*	This study	*yoaD* amplified with primers DH62 and DH63 subcloned into pCKR101 at the KpnI and XbaI sites.
pCKR101-*bcsA*^{WT}	This study	*bcsA* amplified with primers DH36 and DH37 subcloned into pCKR101 at the KpnI and XbaI sites.
pCKR101-*bcsA*^{1}	This study	*bcsA* PilZ *mutant 1* initially amplified with primers DH36&DH45 and DH37&DH44 followed by amplification of first PCR products with primers DH36 and DH37 subcloned into pCKR101 at the KpnI and XbaI sites.
pCKR101-*bcsA*^{2}	This study	*bcsA* PilZ *mutant 2* initially amplified with primers DH36&DH47 and DH37&DH46 followed by amplification of first PCR products with primers DH36 and DH37 subcloned into pCKR101 at the KpnI and XbaI sites.
pFD1	Rubin *et al.* 1999	
pCKR101-*yfiB*	This study	*yfiB* amplified with primers DH168 and DH169 subcloned into pCKR101 at the KpnI and XbaI sites.

19. Depas WH, Hufnagel DA, Lee JS, Blanco LP, Bernstein HC, *et al.* (2013) Iron induces bimodal population development by *Escherichia coli*. Proc Natl Acad Sci U S A 110: 2629-2634.

28. Rubin EJ, Akerley BJ, Novik VN, Lampe DJ, Husson RN, et al. (1999) In vivo transposition of mariner-based elements in enteric bacteria and mycobacteria. Proc Natl Acad Sci U S A 96: 1645-1650.

Primer List

name	description	sequence
DH32	pRJ800 lacZ 16s F	GAT CGG ATC CTA TAC CGA TTG GGA GCA GGT
DH33	pRJ800 lacZ 16s R	GAT CTC TAG AAA AAG TTT GAT GCT CGT GAA TTA AA
DH34	pRJ800 lacZ *adrA* F	GAT CGG ATC CCA AAA GAT GCG CGA ATG TAA TAA TC
DH35	pRJ800 lacZ *adrA* R	GAT CTC TAG ATC AGA AAC AAT TTT CCC AAA TTA TA
DH36	*bcsA* F KpnI pCKR101 SOE	GAT CGG TAC CAT GAG TAT CCT GAC CCG GTG
DH37	*bcsA* R XbaI pCKR101 SOE	GAT CTC TAG ATC ATT GTT GAG CCA AAG CCT
DH44	*bcsA* pilZ pckr101 mut1 C F	GCA AAC AGG TAG ACC GAT CGC ACC G
DH45	*bcsA* pilZ pckr101 mut1 B R	CGG TGC GAT CGG TCT ACC TGT TTG C
DH46	*bcsA* pilZ pckr101 mut2 C F	GCC GAT CGC ACG ACG TGG AGA TGA C
DH47	*bcsA* pilZ pckr101 mut2 B R	GTC ATC TCC ACG TCG TGC GAT CGG C
DH62	*yoaD* F KpnI pckr101	GAT CGG TAC CTT GTC GCC ATA CGC TTA CAG
DH63	*yoaD* R XbaI pckr101	GAT CTC TAG ATT AAC GTA ACG GCA TAA TGG
DH74	prj800 *yoaD* F BamHI	GAT CGG ATC CTG CAG GGC AAG TTC AGG T
DH75	pRJ800 *yoaD* R XbaI	GAT CTC TAG AAC GCC TGA CGC AAC AGT GGA
DH149	UTI89 *yfiN* RS F	TCT TAA TAA GCG CCC CAC GTT TAA AAG AGC ATT ACG CAA CAT CAG TAT GAG TGT AGG CTG GAG CTG CTT C
DH150	UTI89 *yfiN* RS R	AGG TGC TTT ATC ATA TCG ATA TAT CCT TGT TAT CTC ACC AGC TTT TCG GCC ATA TGA ATA TCC TCC TTA G
DH151	UTI89 *yfiR* RS F	TGA AAA AAT ATC GAT GCA TTT CGA GCG AAG ATG GTG AGG ATC CCT GAA TGG TGT AGG CTG GAG CTG CTT C
DH152	UTI89 *yfiR* RS R	CGA GCA TTA AGA CAT CCG GGT TGA CTT TTA CAC CGC TAC GAG ATA AAG CAC ATA TGA ATA TCC TCC TTA G
DH157	UTI89 *yfiB* RS F	CAA GGA TAT ATC GAT ATG ATA AAG CAC CTG CTA GCA CCC CTG ATT TTC ACG TGT AGG CTG GAG CTG CTT C
DH158	UTI89 *yfiB* RS R	GCT GGC CCT TTT TTT GTC TGT TTG CTC GTG TTT TAA GGG GTT GTA ATC ACC ATA TGA ATA TCC TCC TTA G
DH165	UTI89 pckr101 KpnI *yfiR* F	GAT CGG TAC CAT GCG TTT TTC TCA CCG ACT
DH166	UTI89 pckr101 XbaI *yfiR* R	GAT CTC TAG ATT ATC CAT CAT TTT TCT TCC
DH167	UTI89 pckr101 XbaI yfiR R HisT	GAT CTC TAG ATT AGT GAT GGT GAT GGT GAT GTC CAT CAT TTT TCT TCC
DH168	UTI89 pckr101 KpnI *yfiB* F	GAT CGG TAC CAT GAT AAA GCA CCT GCT AGC
DH169	UTI89 pckr101 XbaI *yfiB* R	GAT CTC TAG ATT AAG GGG TTG TAA TCA CCA
WD277	UTI89 *dsbA* RS left	TTC ACG GGC TTT ATG TAA TTT ACA TTG AAT TAT TTT TTC TCG GAC AGA TAG TGT AGG CTG GAG CTG CTT C
WD278	UTI89 *dsbA* RS right	ACC CCC TTT GCA ATT AAC ACC TAT GTA TTA ATC GGA GAG AGT AGA TCA TGC ATA TGA ATA TCC TCC TTA G
WD273	UTI89 *dsbB* RS left	AAA GCG CTC CCG CAG GAG CGC CGA ATG GAT TAG CGA CCG AAC AGG TCA CGC ATA TGA ATA TCC TCC TTA G
WD274	UTI89 *dsbB* RS right	GAA TTG GTT TAA ACT GCG CAC TCT ATG CAT ATT GCA GGG AAA TGA TTA TGG TGT AGG CTG GAG CTG CTT C
WD99	*adrA* Red swap left. Use w/	CTT CTG CCT TTA GCC CCG TCT CTA TAA TTT GGG AAA ATT

	WD100 in UTI89.	GTT TCT GAA TGG TGT AGG CTG GAG CTG CTT C
WD100	adrA Red swap right. Use w/ WD99 in UTI89.	CAG CAA ATC CTG ATG GCT TTT GCC GGA CGT CAG GCC GCC ACT TCG GTG CGC ATA TGA ATA TCC TCC TTA G
WD36	UTI89 *csgD* Red swap primer F. Use w/ pKD4 and WD 37.	CAA TCC AGC GTA AAT AAC GTT TCA TGG CTT TAT CGC CTG AGG TTA TCG TTC ATA TGA ATA TCC TCC TTA
WD37	UTI89 *csgD* Red swap primer R. Use w/ pKD4 and WD36	GAG GCA GCT GTC AGG TGT GCG ATC AAT AAA AAA AGC GGG GTT TCA TCA TGG TGT AGG CTG GAG CTG CTT C
WD6	Red swap F primer for deleting *csgBA* in UTI89.	AAA TAC AGG TTG CGT TAA CAA CCA AGT TGA AAT GAT TTA ATT TCT TAA GTG TGT AGG CTG GAG CTG CTT
WD7	Red swap R primer for deleting *csgBA* in UTI89.	CGA AAA AAA ACA GGG CTT GCG CCC TGT TTC TTT AAT ACA GAG GAT GTA TAT GAA TAT CCT CCT TAG
WD349	*bcsA* UTI89 RS F	TGC CTG TTA AAC TAT TCC GGG CTG AAA ATG CCA GTC GGG AGT GCA TCA TGC ATA TGA ATA TCC TCC TTA G
WD350	*bcsA* UTI89 RS R	AGA ATA TTT TTC TTT TCA TCG CGT TAT CAT CAT TGT TGA GCC AAA GCC TGG TGT AGG CTG GAG CTG CTT C
NDH89	Mariner 1	GGC CAC GCG TGC ACT AGT ACN NNN NNN NNN TAC NG
NDH90	Mariner 2	ATG CAT TTA ATA CTA GCG ACG C
NDH91	Mariner 3	GGC CAC GCG TGC ACT AGT AC
NDH92	Mariner 4	GCG ATC TAT GTG TCA GAC CGG

Strain List

Strain Name	Strain Genotype	Reference	Notes
	UTI89	Mulvey *et al.* 2001	
	UTI89 Δ*csgD*	DePas *et al.* 2013	
	UTI89 Δ*csgBA*	DePas *et al.* 2013	
	UTI89 Δ*bcsA*	DePas *et al.* 2013	
CL-1484	UTI89 Δ*dsbA*	This study	Red Swap mutagenesis of UTI89 with primers WD277 and WD288 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1291	UTI89Δ*dsbB*	This study	Red Swap mutagenesis of UTI89 with primers WD273 and WD274 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1413	UTI89 Δ*dsbB* pRJ800-*adrA*	This study	UTI89 ΔdsbB electroporated with prj800-*adrA*
CL-1414	UTI89 Δ*dsbB* pRJ800-16s	This study	UTI89 ΔdsbB electroporated with prj800-16s
CL-1415	UTI89 Δ*dsbB* pRJ800	This study	UTI89 ΔdsbB electroporated with prj800
	UTI89 pRJ800-*adrA*	DePas *et al.* 2013	
	UTI89 pRJ800-16s	DePas *et al.* 2013	
	UTI89 pRJ800	DePas *et al.* 2013	
CL-1869	UTI89 Δ*dsbB*Δ*csgBA*	This study	Red Swap mutagenesis of UTI89 Δ*dsbB* with primers WD6 and WD7 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1866	UTI89 Δ*dsbB*Δ*bcsA*	This study	Red Swap mutagenesis of UTI89 Δ*dsbB* with primers WD349 and WD350 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1354	UTI89 Δ*csgD*Δ*dsbB*	This study	Red Swap mutagenesis of UTI89 Δ*csgD* with primers WD273 and WD274 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1394	UTI89 Δ*csgD*Δ*dsbB*Δ*bcsA*	This study	Red Swap mutagenesis of UTI89 Δ*csgD*Δ*dsbB* with primers WD349 and WD350 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1543	UTI89 Δ*dsbB*Δ*adrA*	This study	Red Swap mutagenesis of UTI89 Δ*dsbB* with primers WD99 and WD100 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette

CL-1393	UTI89 Δ*csgD*Δ*dsbB*Δ*adrA*	This study	Red Swap mutagenesis of UTI89 Δ*csgD*Δ*dsbB* with primers WD99 and WD100 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1355	UTI89 Δ*csgD*Δ*dsbA*	This study	Red Swap mutagenesis of UTI89 Δ*csgD* with primers WD277 and WD278 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1827	UTI89 Δ*csgD*Δ*dsbB*Δ*yfiN*	This study	Red Swap mutagenesis of UTI89 Δ*csgD*Δ*dsbB* with primers DH149 and DH150 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1825	UTI89 Δ*yfiN*	This study	Red Swap mutagenesis of UTI89 with primers DH149 and DH150 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1826	UTI89 Δ*yfiR*	This study	Red Swap mutagenesis of UTI89 with primers DH151and DH152 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1837	UTI89 Δ*csgD*Δ*yfiR*	This study	Red Swap mutagenesis of UTI89 Δ*csgD* with primers DH151and DH152 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
CL-1828	UTI89 Δ*yfiB*	This study	Red Swap mutagenesis of UTI89 with primers DH157 and DH158 followed by electroporation with the recombinase PCP20 to remove kanamycin cassette
	UTI89 pCKR101	DePas *et al.* 2013	
CL-1840	UTI89 pCKR101-*yfiR*	This study	UTI89 electroporated with pCKR101-*yfiR*
CL-1883	UTI89 pCKR101-*yfiR*^{His}	This study	UTI89 electroporated with pCKR101-*yfiR*^{His}
CL-1646	UTI89 Δ*dsbA* pCKR101	This study	UTI89 Δ*dsbA* electroporated with pCKR101
CL-1884	UTI89 Δ*dsbA* pCKR101-yfiR^{His}	This study	UTI89 Δ*dsbA* electroporated with pCKR101-*yfiR*^{His}
CL-1532	UTI89 Δ*dsbB* pCKR101	This study	UTI89 Δ*dsbB* electroporated with pCKR101
CL-1841	UTI89 Δ*dsbB* pCKR101-*yfiR*	This study	UTI89 Δ*dsbB* electroporated with pCKR101-*yfiR*
CL-1539	UTI89 Δ*csgD*Δ*dsbB* pCKR101	This study	UTI89 Δ*csgD*Δ*dsbB* electroporated with pCKR101
CL-1842	UTI89 Δ*csgD*Δ*dsbB* pCKR101-*yfiR*	This study	UTI89 Δ*csgD*Δ*dsbB* electroporated with pCKR101-*yfiR*
	UTI89 Δ*csgBA*Δ*bcsA*	DePas *et al.* 2013	
CL-1394	UTI89 Δ*csgD*Δ*dsbB*Δ*bcsA*	This study	Red Swap mutagenesis of UTI89 Δ*csgD*Δ*dsbB* with primers DH349 and DH350
CL-1525	UTI89 pckr101-*yoaD*	This study	UTI89 electorporated with pCKR101-*yoaD*
CL-1526	UTI89 Δ*dsbB* pCKR101-*yoaD*	This study	UTI89 Δ*dsbB* electroporated with pCKR101-*yoaD*
CL-1473	UTI89 Δ*csgD*Δ*dsbB*Δ*bcsA* pCKR101-*bcsA*^{WT}	This study	UTI89 electorporated with pCKR101-*bcsA*^{WT}
CL-1471	UTI89 Δ*csgD*Δ*dsbB*Δ*bcsA* pCKR101-*bcsA*^{1}	This study	UTI89 electorporated with pCKR101-*bcsA*^{1}
CL-1472	UTI89 Δ*csgD*Δ*dsbB*Δ*bcsA* pCKR101-*bcsA*^{2}	This study	UTI89 electorporated with pCKR101-*bcsA*^{2}
CL-1470	UTI89 Δ*csgD*Δ*dsbB*Δ*bcsA* pCKR101	This study	UTI89 electroporated with pCKR101
CL-1914	UTI89 pCKR101-*yfiB*	This study	UTI89 electroporated with pCKR101-*yfiB*

77. Mulvey MA, Schilling JD, Hultgren SJ (2001) Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. Infect Immun 69: 4572-4579.

19. Depas WH, Hufnagel DA, Lee JS, Blanco LP, Bernstein HC, *et al.* (2013) Iron induces bimodal population development by *Escherichia coli*. Proc Natl Acad Sci U S A 110: 2629-2634.