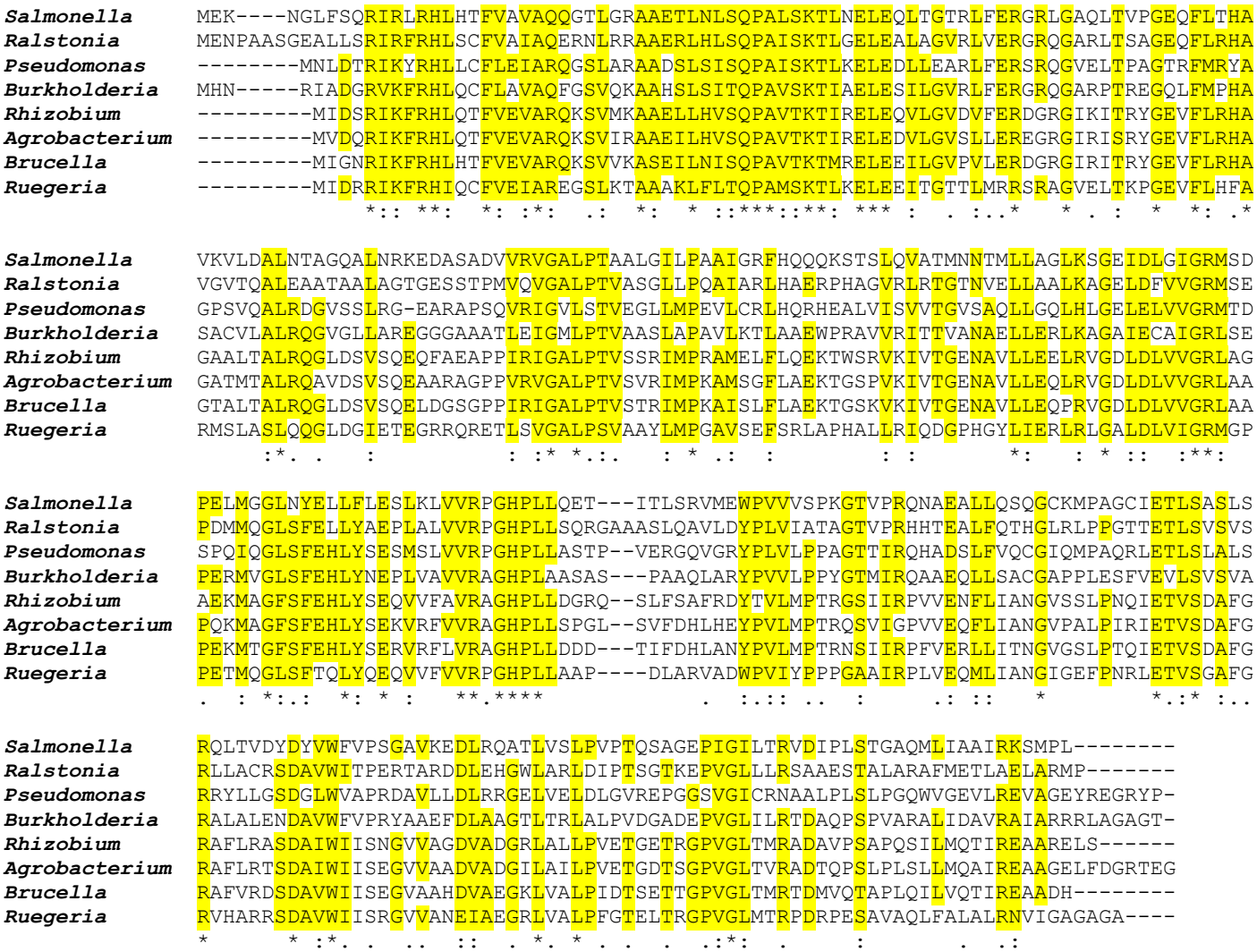
Table S1. DNA primers used in this study.

Name	**Sequence**
Δ*ydcI*	
P1ydcIkd3	GTC TGT TTG AAC GCG GGC GAC TGG GCG CGC AAT TAA CGG TGT GTA GGC TGG AGC TGC TTC
Kd3ydcIp2	GAC AGC CGC TAT CAC AGC GGC ATC GAT TTA CGG ATC GCC GCA TAT GAA TAT CCT CCT TAG TTC C
lacZ fusion	
YdcIendP1	GCGGCTGTCGGAGAATAAACGCGCCCGCGGCTAAAATAGCTGTGTA GGCTGGAGCTGCTTC
YdcItrgP2	CATGATACTGGCGCGACGATGCGATCATCCTTCCTTTAGTCATATG AATATCCTCCTTAGTTCC
Gel shift probes	
For H3:	
5Yhalf	CTTAACGGCAGTTGAATCATCAGTAAA
ydcIprom3	CGCAACCGAATGCGCTGACTAAACAGA
For H5:	
5ydcIprom	ATCTCATCTGCCGTGATGGTGTTCGCC
Yhalf3	CGTGTTAAGCCGCGATCTGGTCGG
For 362-bp:	
5ydcIprom	ATCTCATCTGCCGTGATGGTGTTCGCC
ydcIprom3	CGCAACCGAATGCGCTGACTAAACAGA
For *lacZ*:	
150lacZ5	TGTGCGGCGAGTTGCGTGACTACCTAC
3lacZ150	TTCGACGTTCAGACGTAGTGTGACGCG

RT-PCR

S. typhimurium	
5ydcI	CTGAACGAACTGGAACAACTCACC
ydcI3a	CATCGTATTGTTCATGGTCGCGACCTG
S. typhimurium NCR	
5dcuSint	CCGTGATGGGTAATTTATTTTCATCAGAATTAC
intdcuS3	GAAAACTAATCATAATAGATTAAATATTAGCGCTG
S. typhi	
Ty5	ATGATGAATATCTCCCAGCCGGCGCTATCG
3Ty	CAGGCTCAGAATCGCCTGTGATACGCAGTC
P. mirabilis	
PM5	CCGCAAGGCTAAGCAATTGAATAATTTCATC
3PM	TGCGTAATACGGTGTTACCTGATAATTTGC
S. marcescens	
SER5	GACTGAGCATGGTATTCAGCTTCTCGGTTATGCC
3SER	CATCGTCGTGCTGCGCTTTACGCGCACGTC
K. pneumoniae	
KP5	ATCGACATCGGCATTGGACGTATGTCCGAC
3KP	GCGCGACAGGGAGGCGGAGAGGGTCTCGAT
C. koseri	
CK5	AACCCTTAACCTGAGTCAACCTGCACTC
3CK	CCTGGCCAGCGGTATTAAGCGCATCAAGCAC

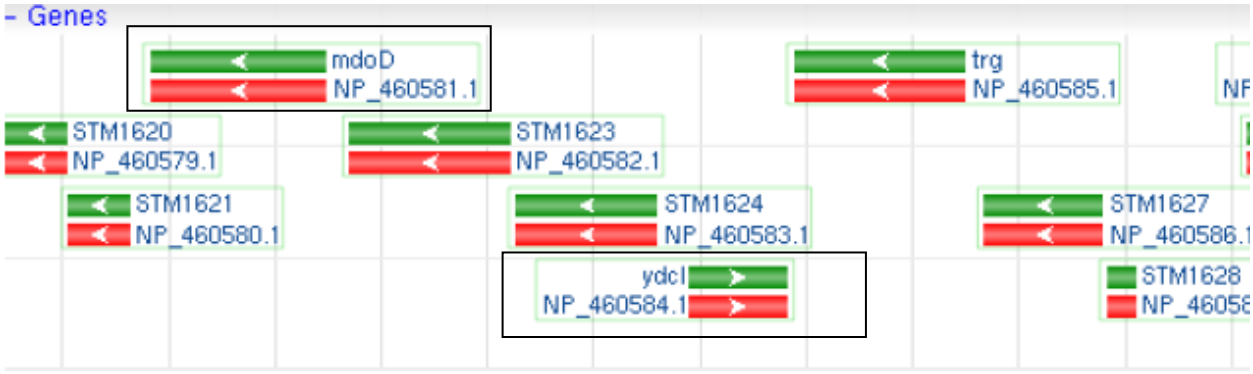
Figure S1.


Salmonella
Ralstonia
Pseudomonas
Burkholderia
Rhizobium
Agrobacterium
Brucella
Ruegeria

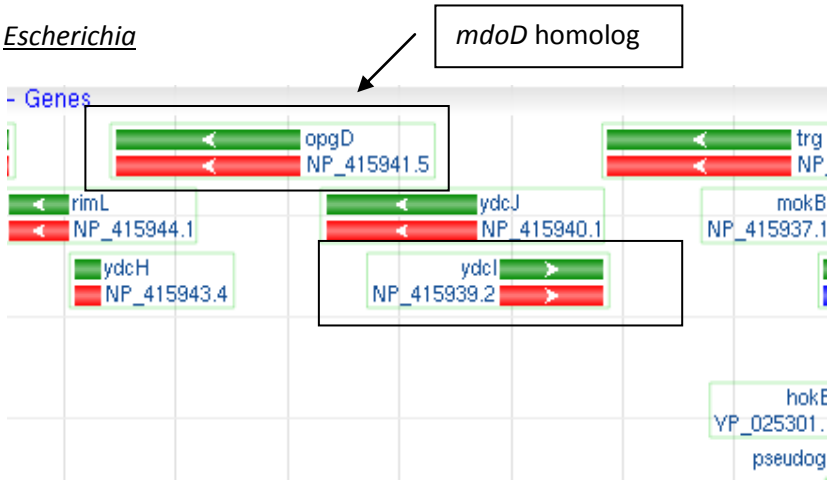
<u>Figure S1.</u> Alignment of the *S. typhimurium* YdcI protein with homologs present in a range of other Gram negative genera more distantly related to *Salmonella* spp. Yellow highlight indicates amino acid identity (in at least five of the proteins). The e-values for the aligned proteins are between 10^{-71} and 10^{-49}.

Figure S2.

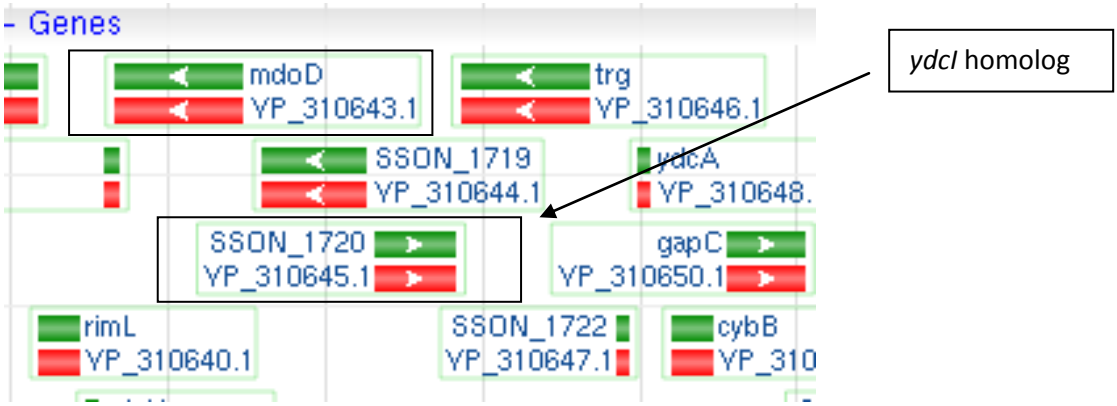
Salmonella



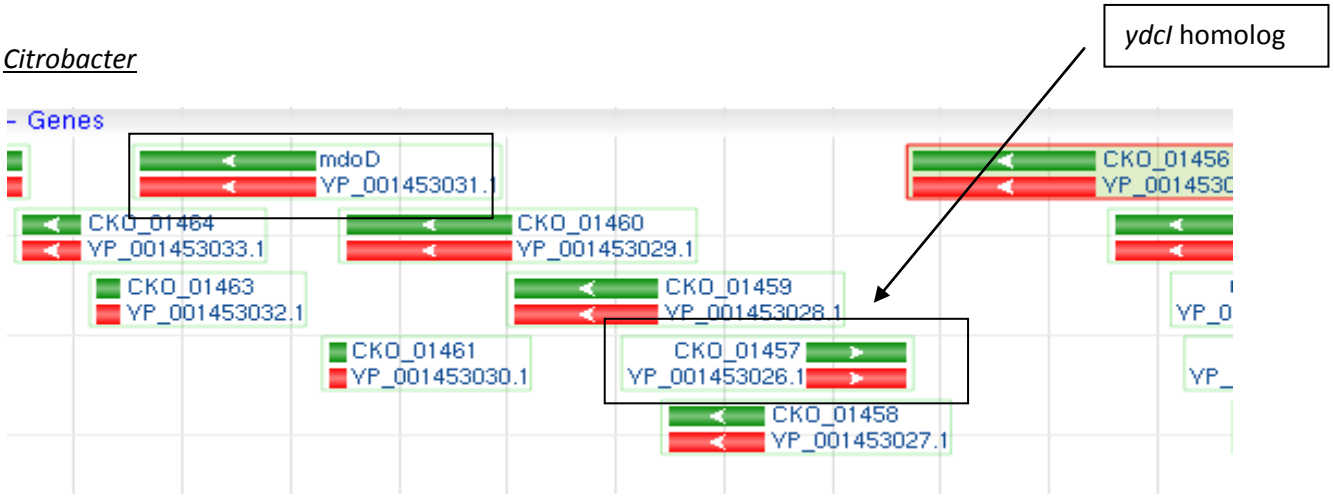
Escherichia



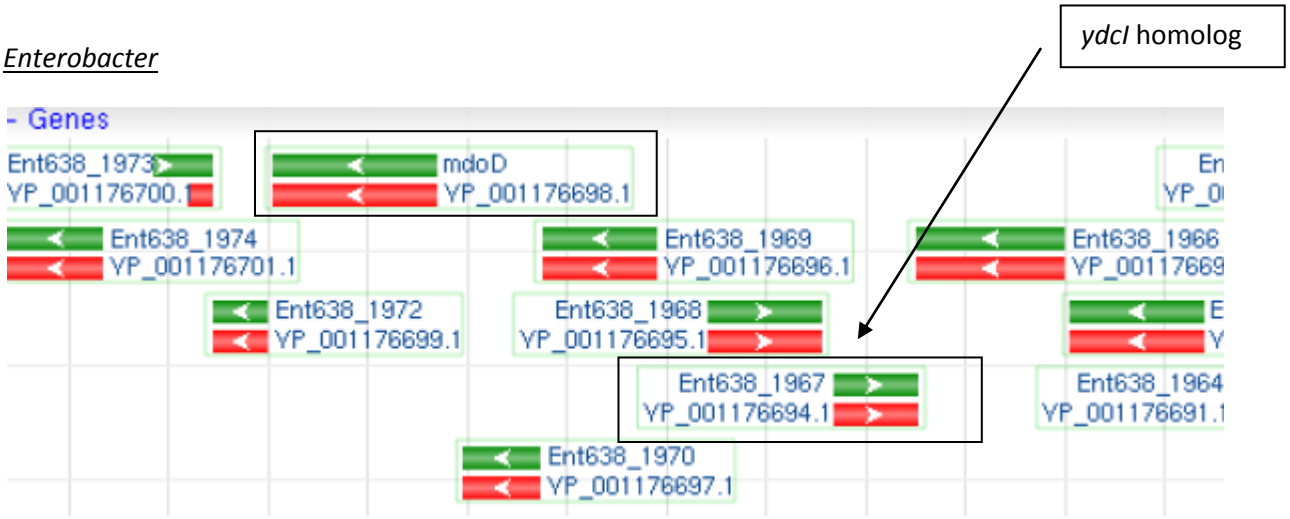
Shigella



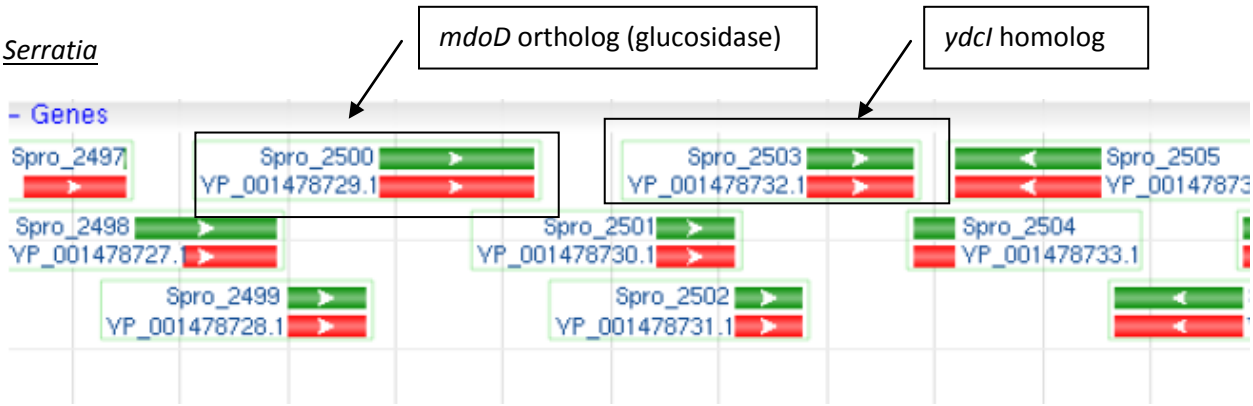
Citrobacter



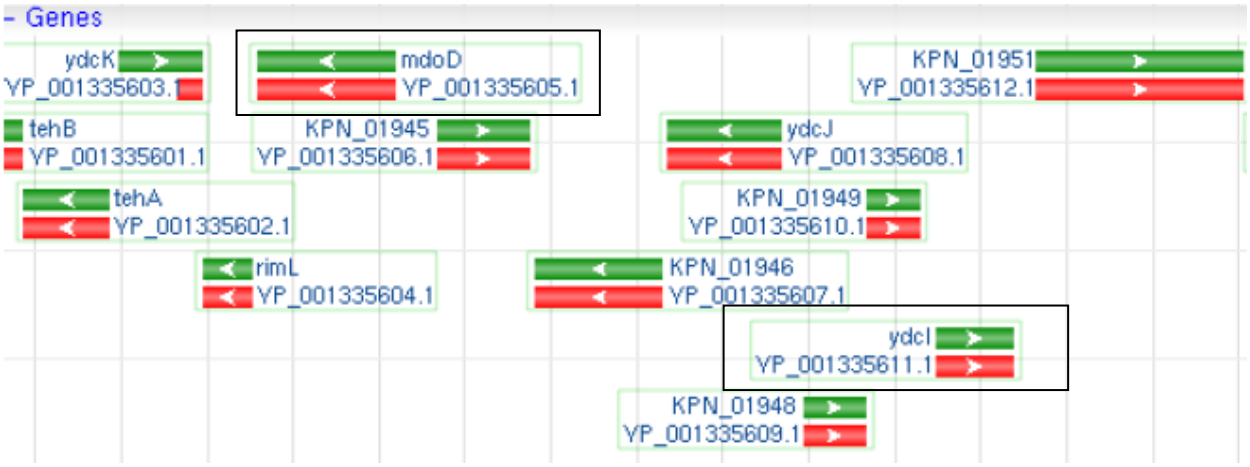
Enterobacter



Serratia



Klebsiella


- Genes
ydcK
YP_001335603.1
mdoD
YP_001335605.1
KPN_01951
YP_001335612.1
tehB
YP_001335601.1
KPN_01945
YP_001335606.1
ydcJ
YP_001335608.1
tehA
YP_001335602.1
KPN_01949
YP_001335610.1
rimL
YP_001335604.1
KPN_01946
YP_001335607.1
ydcI
YP_001335611.1
KPN_01948
YP_001335609.1

<u>Figure S2.</u> Genomic organization of the *ydcI* gene locus in a range of Gram negative genera. Maps of the genes associated with the *ydcI* gene homolog in the genomes of the indicated genera are provided. The *ydcI* genes and the *mdoD* genes are boxed for convenient visual reference.

Figure S3.

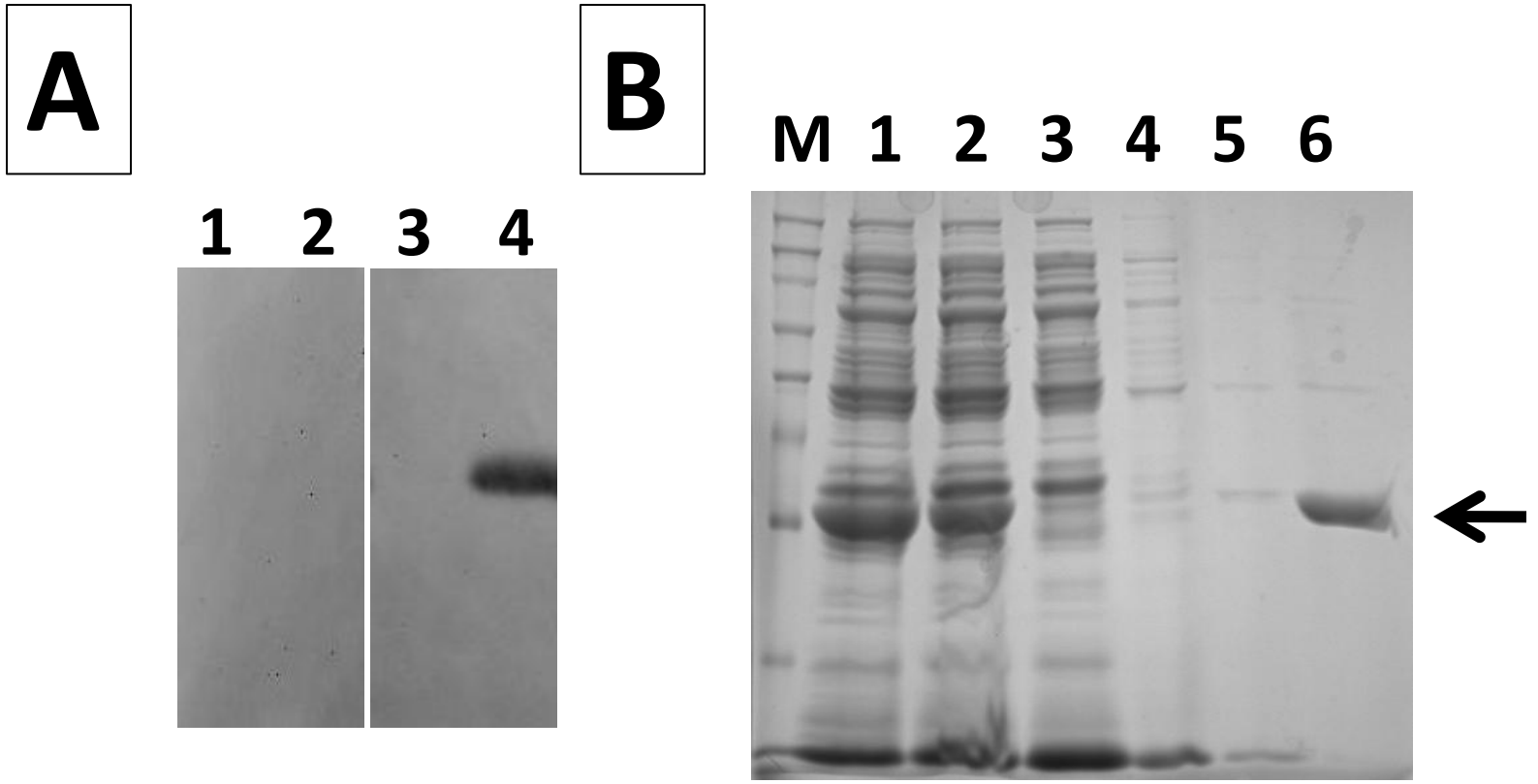


Figure S3. Expression and purification of YdcI protein. Panel A. Broth cultures of *E. coli* TOP10 strains containing pBAD18 or pBAD18 + *ydcI* grown in the presence and absence of arabinose were harvested for total cell protein. A Western blot of these samples was probed with anti-6x-histidine antibodies. Lanes correspond to: 1. pBAD18, - ara; 2. pBAD18, + ara; 3. pBAD18+*ydcI*, - ara; 4. pBAD18+*ydcI*, + ara. Panel B. Coomassie-stained SDS-PAGE gel of samples from purification of 6x-histidine-tagged YdcI from strain TOP10 (pBAD18 + *ydcI*). Lanes: 1. Total cell protein; 2. Soluble protein fraction; 3. Column flow-through; 4. First wash; 5. Second wash; 6. Elution.

Figure S4.

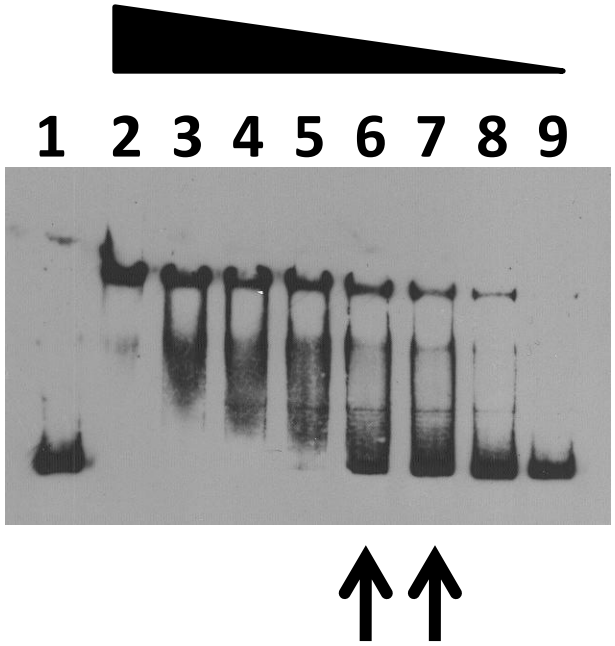


Figure S4. Determination of apparent dissociation constant (Kd) of YdcI protein binding to *ydcI* promoter probe. Lanes: 1. Probe alone; 2-9. Reactions with constant amount of probe (1.6 ng) and 74, 37, 18, 9.2, 4.6, 2.3, 1.2, and 0.6 nM YdcI protein, respectively. Arrows indicate the range at which the likely apparent Kd occurs (between 2.3 and 4.6 nM). The probe is a 362-bp fragment containing the *S. typhimurium ydcI* promoter (genomic coordinates 1714935 – 1714573). The Alphaview software package (Cell Biosciences, Santa Clara, CA) was used for quantification to confirm the apparent Kd range.

Figure S5.

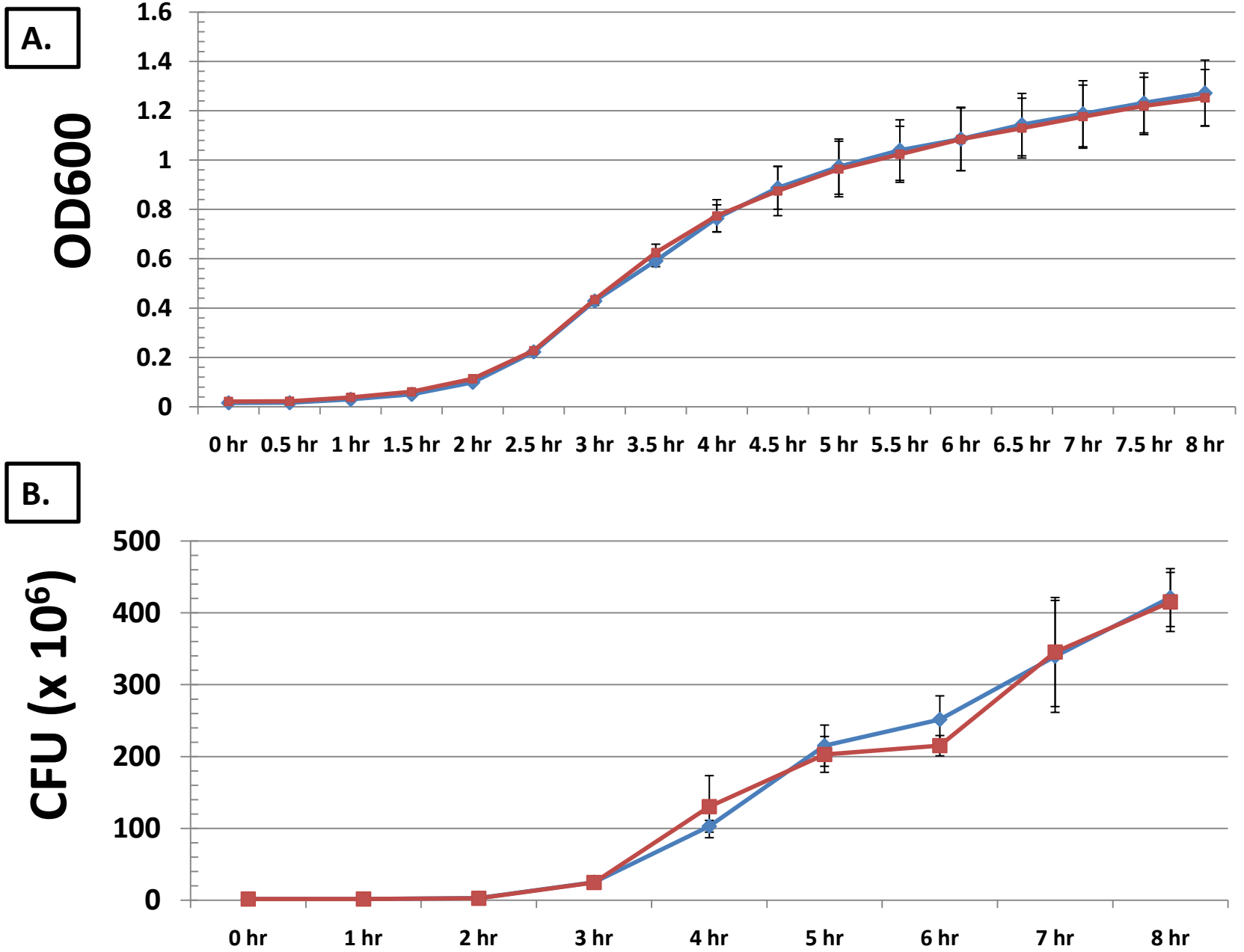
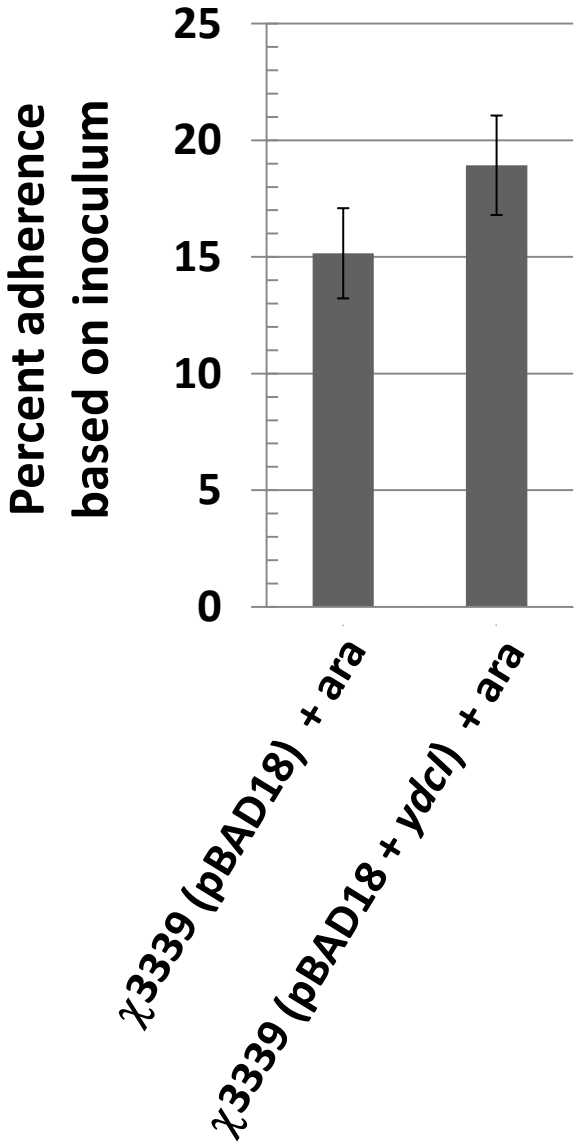

A.
OD600
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 hr 0.5 hr 1 hr 1.5 hr 2 hr 2.5 hr 3 hr 3.5 hr 4 hr 4.5 hr 5 hr 5.5 hr 6 hr 6.5 hr 7 hr 7.5 hr 8 hr
B.
CFU (x 10^6)
500
400
300
200
100
0
0 hr 1 hr 2 hr 3 hr 4 hr 5 hr 6 hr 7 hr 8 hr

Figure S5. Growth curves of isogenic *S. typhimurium* WT and Δ*ydcI* strains. Cultures of WT and Δ*ydcI* strains were diluted into fresh LB media, grown shaking at 37 degrees C, and sampled for growth at the indicated time points for OD600 (Panel A) and CFU (Panel B) measurements. The data represent the mean and standard deviation from three independent cultures. The red and blue lines indicate the WT and Δ*ydcI* strains, respectively.

Figure S6.


Percent adherence based on inoculum
25
20
15
10
5
0
χ3339 (pBAD18) + ara
χ3339 (pBAD18 + ydcI) + ara

<u>Figure S6.</u> Attachment of *S. typhimurium* χ3339 (pBAD18) and χ3339 (pBAD18 + *ydcI*) strains to Int407 cells. Samples of the indicated strains were added to culture wells containing Int407 cells (at MOI of approximately 30) and allow to adhere for 30 minutes. Following washes, cells were harvested using deoxycholate, serial diluted in PBS, and plated for CFU counts. The percent bacteria adhering to the tissue culture cells based on inoculum is presented. Data represent the mean and standard deviation from four independent trials each performed in triplicate wells.

Figure S7.

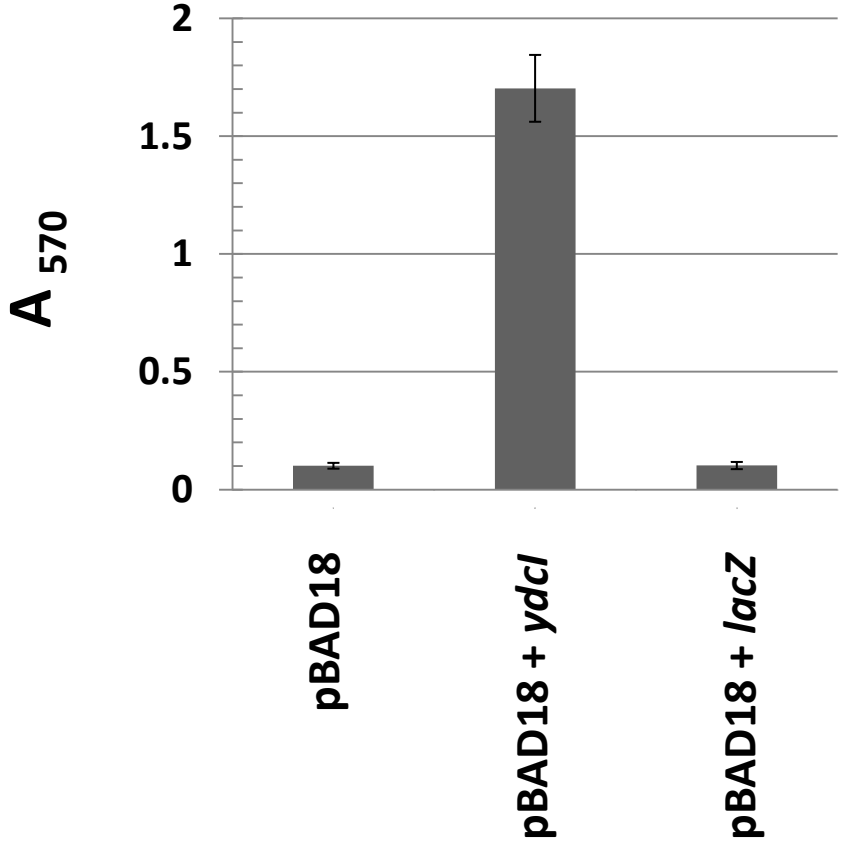


Figure S7. Quantitation of biofilm formation. Separate cultures of *S. typhimurium* strain χ3339 containing the indicated plasmids were grown for biofilm as indicated in Figure 9 of the main text. After washing with PBS, the culture flasks were stained with crystal violet, and then the sides of the flasks were washed with 33% acetic acid as described previously in the text. Absorbance of the sample was determined at 570 nm wavelength and plotted. The results are obtained from three or four independent experimental cultures for each strain.