

# The conserved DNA-binding protein WhiA is involved in cell division in *Bacillus subtilis*

## Supplemental Material

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Table S1. Bacterial strains and plasmids used in this study.

<i>B. subtilis</i>	Relevant genotype	Source & comment
168	<i>trpC2</i> (0168 ED)	wild-type for this study
1283	<i>trpC2</i> $\Delta$ <i>noc::spc</i>	(1)
1356	<i>trpC2</i> $\Delta$ <i>zapA-yshB::tet</i>	(2)
1801	<i>trpC2</i> chr:: pJSlZDpble ( $P_{spac^-}$ <i>ftsZ ble</i> )	(3)
2020	<i>trpC2 amyE::</i> ( $P_{xyr^-}$ <i>gfpmut1-ftsZ spc</i> )	J. Sievers (unpublished)
3309	<i>trpC2 minCD::kan</i>	Laboratory stock
4701	<i>trpC2 amyE::P<sub>xyr^-</sub>-noc-yfpmut1</i>	(4)
BFA2863	<i>trpC2</i> $\Delta$ <i>sepF::ery</i> ( <i>pMutin4YImF</i> )	(5)
KS6	<i>trpC2</i> $\Delta$ <i>zapA-yshB::tet</i>	168::chr. DNA 1356
KS44	<i>trpC2</i> $\Delta$ <i>ezrA::cat</i>	D. Claessen, unpublished
KS50	<i>trpC2</i> $\Delta$ <i>lacA::cat</i> $\Delta$ <i>zapA-yshB::tet</i> pLOSS-zapA	This work
KS162	<i>trpC2</i> $\Delta$ <i>zapA-yshB::tet</i> $P_{spac^-}$ <i>ftsZ ble</i>	KS6::chr. DNA 1801
KS207	<i>trpC2</i> $\Delta$ <i>yvcL::pMutin4YvcL</i> ( <i>ery</i> )	This work
KS267	<i>trpC2</i> $\Delta$ <i>yvcL::TnYLB-1</i> ( <i>kan</i> )	This work, transposon disruption
KS268	<i>trpC2 P<sub>spac^-</sub>ftsZ ble</i>	168::chr. DNA 1801
KS338	<i>trpC2</i> $\Delta$ <i>minCD::kan</i>	168::chr. DNA 3309
KS341	<i>trpC2</i> $\Delta$ <i>yvcL::TnYLB-1</i> ( <i>kan</i> ) $\Delta$ <i>sepF::ery</i>	KS267::chr. DNA BFA2863
KS344	<i>trpC2</i> $\Delta$ <i>yvcL::TnYLB-1</i> ( <i>kan</i> ) $\Delta$ <i>ezrA::cat</i>	KS267::chr. DNA KS44
KS345	<i>trpC2</i> $\Delta$ <i>noc::spc</i>	168::1283
KS354	<i>trpC2</i> $\Delta$ <i>yvcL::TnYLB-1</i> ( <i>kan</i> ) $\Delta$ <i>noc::spc</i>	KS267::chr. DNA KS345
KS356	<i>trpC2</i> $\Delta$ <i>yvcL::ery</i> $\Delta$ <i>minCD::kan</i>	KS207::chr. DNA KS338
KS396	<i>trpC2 amyE::P<sub>xyr^-</sub>-yvcL spc</i>	This work; 168:: pSG1729YvcLAGFP
KS400	<i>trpC2</i> $\Delta$ <i>yvcL::kan</i>	This work
KS438	<i>trpC2 yvcL::P<sub>spac^-</sub>-yvcL ery</i>	This work
KS696	<i>trpC2 yvcL</i>	This work
KS742	<i>trpC2</i> $\Delta$ <i>lacA::cat</i> $\Delta$ <i>zapA-yshB::tet</i> $\Delta$ <i>yvcL</i> pLOSS-YvcL	This work; $\Delta$ <i>yvcL</i> originated from KS696
KS748	<i>trpC2 yvcL P<sub>spac^-</sub>ftsZ ble</i>	KS696::chr. DNA 1801
KS754	<i>trpC2 yvcL::P<sub>spac^-</sub>-yvcL ery</i> $\Delta$ <i>zapA-yshB::tet</i> <i>amyE::P<sub>xyr^-</sub>-gfp-ftsZ</i> ( <i>spc</i> ) pMAP65 ( <i>lacI, kan</i> )	This work, the $P_{xyr^-}$ - <i>gfp-ftsZ</i> fusion originates from 2020
KS859	<i>trpC2 yvcL::P<sub>spac^-</sub>-yvcL ery</i> $\Delta$ <i>zapA-yshB::tet</i> <i>aprE::lacI spc</i>	KS891::chr. DNA KS6
KS891	<i>trpC2 yvcL::P<sub>spac^-</sub>-yvcL ery</i> <i>aprE::lacI spc</i>	KS438::pAPNC213
KS902	<i>trpC2 gtaB::TnYLB-1 kan</i> $\Delta$ <i>yvcL::ery</i>	This work
KS903	<i>trpC2 pgcA::TnYLB-1 kan</i> $\Delta$ <i>yvcL::ery</i>	This work
KS1015	<i>trpC2</i> $\Delta$ <i>yvcL::ery</i> $\Delta$ <i>ugtP::neo</i>	KS207::chr. DNA PG237, this work
PG8	<i>trpC2 amyE::P<sub>xyr^-</sub>-ftsZ cat</i>	168::chr. DNA YK059
PG237	<i>trpC2</i> $\Delta$ <i>ugtP::neo</i>	Pamela Gamba, unpublished
PG725	<i>trpC2 amyE::P<sub>xyr^-</sub>-noc-yfpmut1</i>	168::4701
PG727	<i>trpC2 amyE::P<sub>xyr^-</sub>-noc-yfpmut1</i> $\Delta$ <i>yvcL::kan</i>	KS400::4701
PG732	<i>trpC2 amyE::P<sub>xyr^-</sub>-mgfpmut1-yvcL spc</i>	168::pSG1729YvcL(mGFP), this work
PG735	<i>trpC2 amyE::P<sub>xyr^-</sub>-ftsZ cat</i> $\Delta$ <i>zapA-yshB::tet</i>	PG8::chr. DNA KS6
PG736	<i>trpC2</i> $\Delta$ <i>yvcL::kan</i> <i>amyE::P<sub>xyr^-</sub>-mgfpmut1-yvcL</i> <i>spc</i>	KS400::chr. DNA PG732
PG737	<i>trpC2 amyE::P<sub>xyr^-</sub>-ftsZ cat</i> $\Delta$ <i>yvcL::kan</i>	PG8::chr. DNA KS400
PG738	<i>trpC2 amyE::P<sub>xyr^-</sub>-ftsZ cat</i> $\Delta$ <i>zapA-yshB::tet</i> $\Delta$ <i>yvcL::kan</i>	PG735::chr. DNA KS400
PG739	<i>trpC2</i> $\Delta$ <i>zapA-yshB::tet</i> $\Delta$ <i>noc::spc</i>	KS6::KS345
PG740	<i>trpC2</i> $\Delta$ <i>zapA-yshB::tet</i> $\Delta$ <i>minCD::kan</i>	KS6::KS338
YK059	CRK6000 <i>amyE::P<sub>xyr^-</sub>-ftsZ cat</i>	(6)

<i>E. coli</i>	Relevant genotype	Source & comment
XL1Blue	<i>recA1 endA1 gyrA96 thi1 hsdR17 supE44 relA1</i> <i>lac</i> [F' <i>proA+B+lacIq</i> $\Delta$ M15 Tn10 (TetR)]	Stratagene
KS432	XL1Blue::pQE60EYvcL	This work

Plasmid	Relevant genotype/comment	Source & comment
pAPNC213	<i>bla aprE5' spc lacI P<sub>spac^-</sub>-mcs aprE3'</i>	(7)
pBEST501	bearing the kanamycin cassette	(8)
pQE60E	derived from pQE60 (Qiagen), expression vector for C-terminal His-6 fusions	L. Hamoen, unpublished
pQE60EYvcL	pQE60E containing <i>yvcL-his6</i>	This work
pLOSS*	<i>bla spc P<sub>spac^-</sub>-mcs P<sub>divIVA</sub>-lacZ lacI rep<sub>pLS20</sub></i> (GA $\rightarrow$ CC)	(9)
pLOSS-zapA	pLOSS* containing <i>zapA-yshB</i>	This work
pLOSS-YvcL	pLOSS* containing <i>yvcL</i>	This work
pMAP65	pUB110 $P_{pen^-}$ - <i>lacI</i>	(10)
pMarB	<i>bla erm P<sub>ctc</sub>-Himar1 kan</i> (TnYLB-1)	(11)

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pMutin4	<i>bla erm lacZ lacI</i>	(12)
pMutin4YvcL	integrates into <i>yvcL</i> gene	This work
pMutin4YvcLKO	pMutin4 containing 3' fragment of <i>yvcK</i> and 5' fragment of <i>yvcL</i>	This work
pMut4YKO	pMutin4YvcLKO introducing a stop codon and <i>EcoRI</i> site into <i>yvcL</i>	This work.
pMutYvcL	used for construction of an <i>yvcL-crh-yvcN</i> conditional mutant	This work
pSG1729	<i>bla amyE3' spc P<sub>xyr</sub>-gfpmut1' amyE5'</i>	(13)
pSG1729YvcL	<i>bla amyE3' spc P<sub>xyr</sub>-gfpmut1-yvcL amyE5'</i>	This work
pSG1729YvcL(mGFP)	<i>bla amyE3' spc P<sub>xyr</sub>-mgfpmut1-yvcL amyE5'</i>	This work
pSG1729YvcLΔGFP	<i>bla amyE3' spc P<sub>xyr</sub>-yvcL amyE5'</i>	This work

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Table S2. Oligonucleotides used in this study.

Name	Restriction site	Sequence (5'-3')	Reference/ used for
HS410		CCTGTCCACACAATCTAAACTTTTCGAAAGATCC C	A206K mutation in GFP coding sequence
HS411		GGGATCTTTTCGAAAGTTTAGATTGTGTGGACAG G	A206K mutation in GFP coding sequence
km3	<i>Bam</i> HI	GGGGGATCCAAGACGAAGAGGATGAAG	<i>kan</i> <sup>R</sup> amplification
km4	<i>Eco</i> RI	CCCGAATTCAGAGTATGGACAGTTGCG	<i>kan</i> <sup>R</sup> amplification
KS80	-	GTACAGGTCTTTCTGTATTG	KS400 construction
KS83	-	GGCGCTCTGACATGACCATC	KS400 construction
KS84	<i>Eco</i> RI	CGGACAGAATTCACCGTCACTTTAAAATAAC	KS400 construction
KS89	<i>Bam</i> HI	TGAGGTGGATCCATGTCATTTGCATCAGAAA	pQE60E construction
KS94	<i>Hind</i> III	GCTAAGCTTGCATTTCCCAATTTGCTC	pMut <sub>i</sub> YvcL construction
KS95	<i>Bam</i> HI	GATGGATCCCTCATTTCAAGGCTTCATTC	pMut <sub>i</sub> YvcL, KS400 construction
KS97	<i>Bam</i> HI	GATGGATCCTTACGGTTATTTTAAAGTG	pMut <sub>i</sub> Crh, pLOSS-YvcL construction
KS98	<i>Xho</i> I	GATCCTCGAGTAAAGTGACGGTTTGCCCTG	pSG1729YvcLΔGFP construction
KS99	<i>Bgl</i> II	GATCAGATCTTAAAGTGACGGTTTGCCCTG	pQE60E construction
KS120		AATTCTGATAACTGGAAGTGAAGGACTG	pMut4YKO construction
KS121		CCAGTTATCAGAATTTCTTTTTTTGTTTCTGATG	pMut4YKO construction
KS128	<i>No</i> cI	ACTGAAAGCGGCCGCCCTTGAAATGAGGTGGC	pLOSS-YvcL construction
KS185		AATGGAGACTGGATTGCTGTAG	<i>xynD</i> qPCR
KS186		TTACCGTCTGCACTGTCGAG	<i>xynD</i> qPCR
KS187		ATACGCGCAGCATCTTAACG	<i>ynfF</i> qPCR
KS188		GTATTTCTTGCGGCTCCAC	<i>ynfF</i> qPCR
KS198		TCTGAGCCAGAACCTGATCC	<i>spoVID</i> qPCR
KS199		AGTCTCCGCTGGAGAGTCTG	<i>spoVID</i> qPCR
KS200		GATACTAAATCCGCCGGAAC	<i>acoC</i> qPCR
KS201		TCAAATGTCAGGCTGAGTGG	<i>acoC</i> qPCR
OIPCR1		GCTTGATAAATTCTATCATAATTG	(11)
OIPCR2		AGGGAATCATTGAAGGTTGG	(11)
OIPCR3		GCATTTAATACTAGCGACGCC	(11)
STG101		ATACCGCGGAGATACCGAG	<i>scpB</i> Fw qPCR
STG102		TTTGACGGCACTTCAATCAG	<i>scpB</i> Rw qPCR
STG410		TTGACGACAAGCGTGAAAAG	<i>rplC</i> Fw qPCR
STG411		TTcatACGcatCcatTTCCA	<i>rplC</i> Rw qPCR
<i>yshA</i> -F	<i>No</i> cI	GATGCGGCCGCCACTTTTTCGCTGTATATACC	pLOSS-ZapA construction
<i>yshB</i> -R	<i>Bam</i> HI	GATGGATCCGACGTTACATATGTTCCATC	pLOSS-ZapA construction
<i>yvcL</i> -C5	<i>Kpn</i> I	GCCTTGGGTACCGGTGGCTATATGTCATTTG	pSG1729YvcLΔGFP construction
<i>yvcL</i> -F1	<i>Hind</i> III	GCTAAGCTTAAACCGCCATCTCGTACTC	pMut <sub>i</sub> 4YvcL construction
<i>yvcL</i> -R1	<i>Bam</i> HI	GATGGATCCAATTGACGAGGCGGTTGACC	pMut <sub>i</sub> 4YvcL construction
<i>yvcL</i> -N5	<i>Hind</i> III	GCCTTGAAGCTTGGTGGCTATATGTCATTTG	pSG1729YvcL construction
<i>yvcL</i> -N3	<i>Eco</i> RI	GAACCTGAATTCTCTGTTGAACCATAAAGATC	pSG1729YvcL construction

Table S3. Sporulation efficiency.

<b>Strain</b>	<b>Genotype</b>	<b>Total cell count</b>	<b># Phase bright spores</b>	<b>% Sporulation</b>
168	wild type	2997	1988	66.3
KS207	$\Delta yvcL::ery$	2536	1233	48.6
KS696	$\Delta yvcL$	1933	808	41.8

The sporulation efficiencies are calculated from the number of phase bright spores relative to the total number of cells. Cultures were grown in DSM medium for 24 hours at 37°C (two biological and two technical replicates). Spore and cell numbers were determined by light microscopy.

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Bacsu/1-316  1  -----MSFASETKKELTNLEVKDCCI -NAELSALIRMNGALSFTNRHLVLDVQTENAAIARRIYTLIK 62
Staac/1-314  1  -----MSFASEMKNELTRIDVDEMNA-KAELSALIRMNGALSLSNQQFVINVTENATTARRIYSLIK 62
Strp2/1-303  1  -----MSFTVAVKEEILG---QHHL-SWHELSAIIKMSGSIGLSTSGLTLSVVVTENAKLARHLYESFL 59
Strco/1-328  1  -----MTAAVKSEISQLPVTRTCCRKAEVSAVLRFAAGLHLVSGRIVIEAELDTGNAARRLKRDI 61
Myctu/1-325  1  -----MTTDVKDEL SRLVVKSVSARRA EVTSLLRFAAGLHIVGGRVVVEAELDLGSIARRLRKEIF 61
Thema/1-295  1  MVSLLRRTFSEEIK EELVNVVFGSREEVISELLGFIKARGLDVKSRHIVFSLHSFAAS--RRLNLMK 67

Bacsu/1-316  63  KQYDVSVLELVRKKMRLKKNNVYIVRFSENAKALEDLKILGENFVFRSISKELVK-KRCKRSYMRG 130
Staac/1-314  63  RVFNVEVELVRKKMKLKNNIYICRTKMKAKELDELGILKD-GIFTHEIDHSMIQ-DDEMRRSYLRG 129
Strp2/1-303  60  HFYEIKSEIRHHQSRNLKKNRVYTVFTDEKVVQDLSDLHLADSFVLEGTIDEAII-S-DEEAGRAYLCG 127
Strco/1-328  62  EIFGHSSLEIVMAPGGRRGSRFVVRVAVGGDQAROTGLVDGRGRPIRGLPPQVVSATCDAAEAARWG 130
Myctu/1-325  62  ELVGYTAVVHVLASGIRKSTRYVLRVANDGEALAROTGLLDMRGRPVRLPAQVVGGSIDDAEAARWG 130
Thema/1-295  68  YLSKPVSEIIVEKSHNKKRYIKITA EYSESFMIPEPFDVALFVDFLR-----G 117

Bacsu/1-316  131  AFLAGGSVNNPETSSYHLEIFSLYKEHNDSLCDLLNEFQINSKTLERKKGYITYLKEAEKITEFNVIG 199
Staac/1-314  130  AFLAGGSVNNPETSSYHLEIFSQNESHAEGTLKLMNSYENAKHLERKKGSI TYLKEAEKISDFLSLIG 198
Strp2/1-303  128  AFLANGSIRDPESGKYQLEISSVYLDHAQGIASLLQOFLDAKVLERKKGAVTYLQRAEDIMDFLIVIG 196
Strco/1-328  131  AFLAHGSLTEPGRS-SSLEVTCPGPEAALALVGAARRLSIPAKARVVRGVDVVVRDGDAGALLTRLG 198
Myctu/1-325  131  AFLAHGSLTEPGRS-SALEVSCPGPEAALALVGAARRLGVGAKARVVRGADR VVVRDGEAGALLTRMG 198
Thema/1-295  118  LFLSGSSMTNRYHYHLEINLFEETLALTRKSLKDFFINAGIILRNTRKLYIKSIDLVLEAIG 186

Bacsu/1-316  200  AHNSLLRFEDVRIVRDMRNSVNRLVNCETANLNKTI GASLRQVENIKYIDERIGLEALFEKLRREI AQLR 268
Staac/1-314  199  GYQALLKFEDVRIVRDMRNSVNRLVNCETANLNKTVSAAMKQVESIKLIDKEIGIENLPDRLREIARIR 267
Strp2/1-303  197  AMQARDDFERVKILRETRNDLNRANNAETANIARTVSAMKTIINISKIKDIMGLENLPDLQEV AQLR 265
Strco/1-328  199  AHDSVLAWEEERLREVRATANRLANFDDANLRRSARAAVAAGARVQRALEILADD-VPEHLAAAGRLR 266
Myctu/1-325  199  AQDTRLVWEERLREVRATANRLANFDDANLRRSARAAVAAGARVQRALEILGDT-VPEHLASAGKLR 266
Thema/1-295  187  VQRKLEEIDRIVTEKVI GDNRTVNFIEANAIRTANSTARQIRAI ELIKENMGLENLPDLRRVALVR 255

Bacsu/1-316  269  IDYQEVTLKELGEMVASGKISKSGINHRLRKLDEIAEQLRTGQTVTLK----- 316
Staac/1-314  268  VEHQEISLKELGEMVSTGPI SKSGVNHRLRKLNDLADKIRNGEQIEL----- 314
Strp2/1-303  266  IQHPDYSIQQLADSLST-PLTKSGVNHRLRKLINKI ADEL----- 303
Strco/1-328  267  MEHKQASLEELGALADP-PLTKDAVAGRI RRLLAMADKRASDLGIAGTDANLGEEELADNLVG 328
Myctu/1-325  267  VEHRQASLEELGRLADP-PMTKD AVAGRI RRLLSMADRKAKVDGIPDTESVVT PDLLEDA--- 325
Thema/1-295  256  LRNKELSLRELGKKNL---TKSQIYSKLRKI KIIAERFGDVK----- 295

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Fig. S1. Sequence alignment of WhiA-like proteins. The protein sequences were aligned using ClustalW and the alignment was coloured using JalView programme (14). WhiA-like proteins from the following organisms were aligned: (*Bacsu*) *Bacillus subtilis*, (*Staac*) *Staphylococcus aureus*, (*Strp2*) *Streptococcus pneumoniae*; (*Strco*) *Streptomyces coelicolor*; (*Myctu*) *Mycobacterium tuberculosis*, and (*Thema*) *Thermotoga maritima*. Conserved residues are coloured, with the least variable conserved residues in red background.

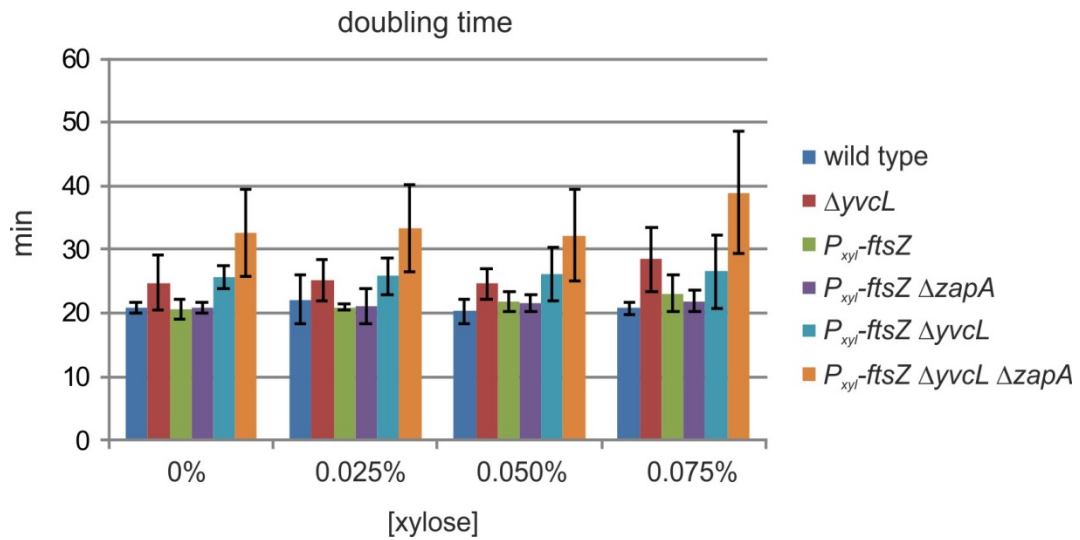


Fig. S2. Growth rates of *yvcL* mutants. Cells of strains: 168 (wild type), KS400 ( $\Delta yvcL$ ), PG8 ( $P_{xyl-ftsZ}$ ), PG735 ( $P_{xyl-ftsZ} \Delta zapA$ ), PG737 ( $P_{xyl-ftsZ} \Delta yvcL$ ), and PG738 ( $P_{xyl-ftsZ} \Delta zapA \Delta yvcL$ ) were initially grown in LB medium with 0.05% xylose, then diluted and grown in LB medium supplemented with increasing concentrations of xylose at 37°C in a plate reader. The doubling times and standard deviations were calculated from three independent experiments.

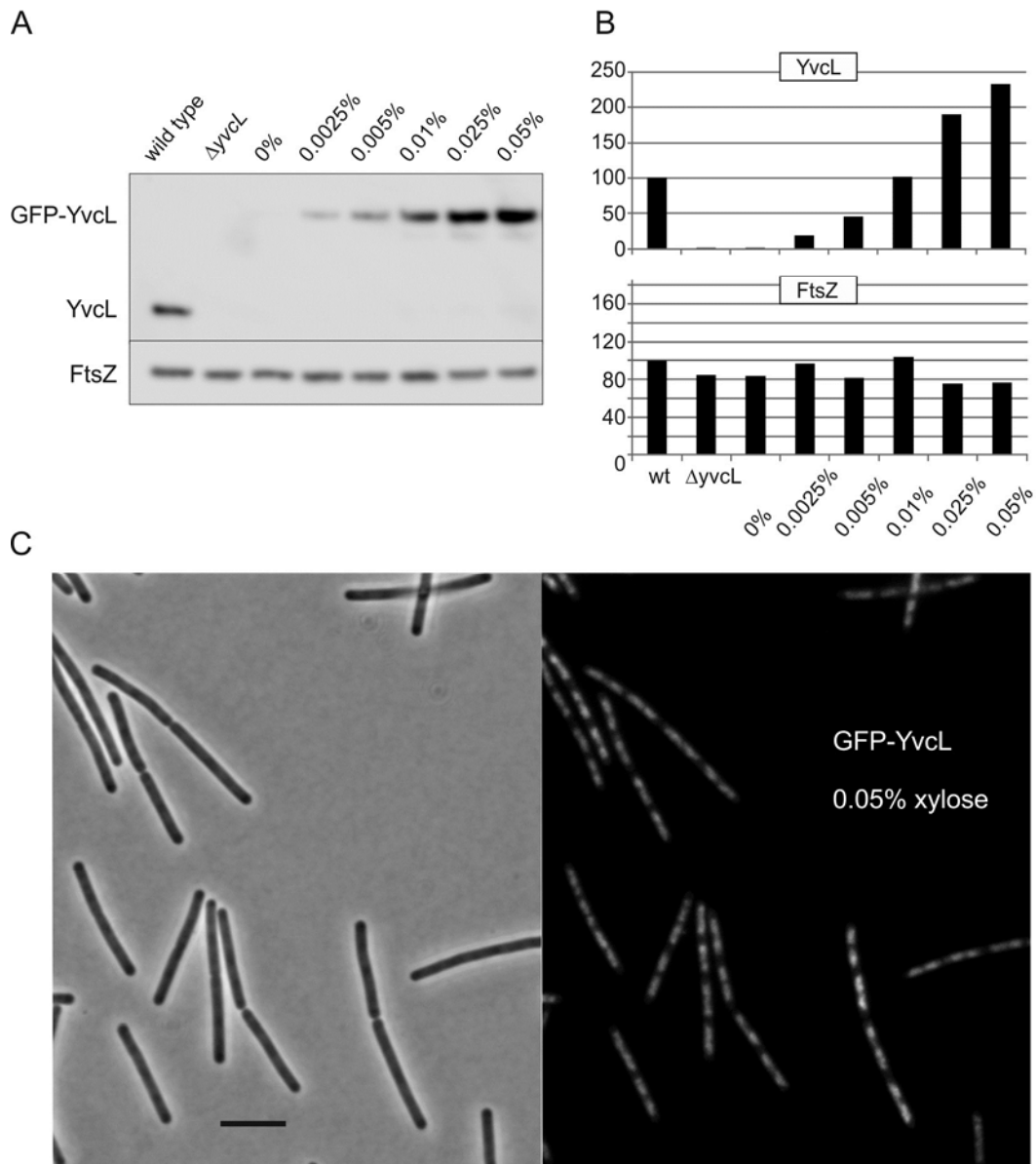


Fig. S3. GFP-YvcL expression levels and localization. (A) Western blot analysis using YvcL antibodies to determine cellular YvcL and GFP-YvcL levels. Strains 168 (wild type),  $\Delta yvcL$ , and PG736 ( $P_{xyI}$ -*mgfp-yvcL*) were grown in LB medium at 30°C. In case of PG736, different concentrations of xylose were added (0% up to 0.05%). FtsZ immunodetection was used as internal control. (B) Band intensities (arbitrary units) of the Western blot in (A). (C) Fluorescence microscopy image of strain PG736 grown in LB medium supplemented with 0.05% xylose at 30°C. Scale bar 5  $\mu$ m.



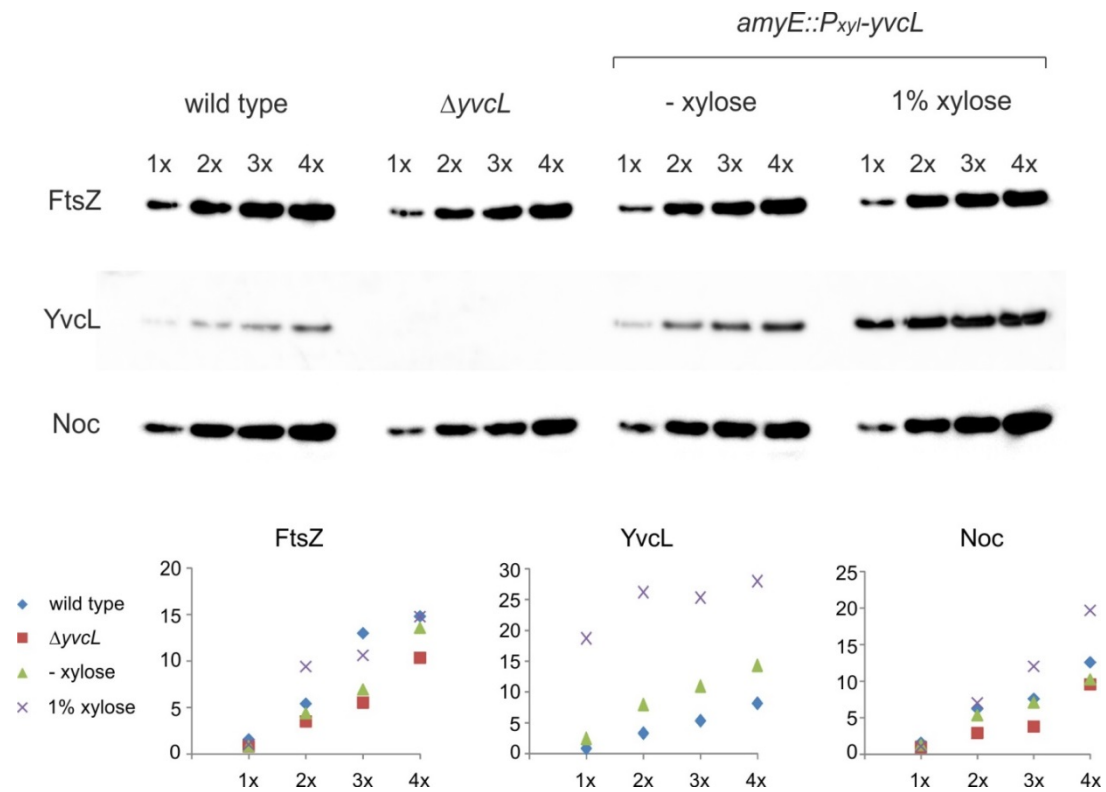


Fig. S4. FtsZ levels in  $\Delta yvcL$  and YvcL-overproduction strains. Western blots indicating FtsZ, YvcL and Noc levels in wild type, *yvcL* mutant strain, and a strain overexpressing YvcL are shown. Samples were diluted (1 to 4 fold) before loading onto the protein gel to facilitate quantification. Graphs presenting the intensities (arbitrary units) of the protein bands are shown below. Strains 168 (wild type), KS400 ( $\Delta yvcL$ ), and KS396 (*P<sub>xyI</sub>-yvcL*) were grown to  $OD_{600} \sim 0.4$  in LB medium at 37°C. Protein concentrations of cell extracts were measured by Bradford protein assay, and immunodetection of Noc was used as internal control.

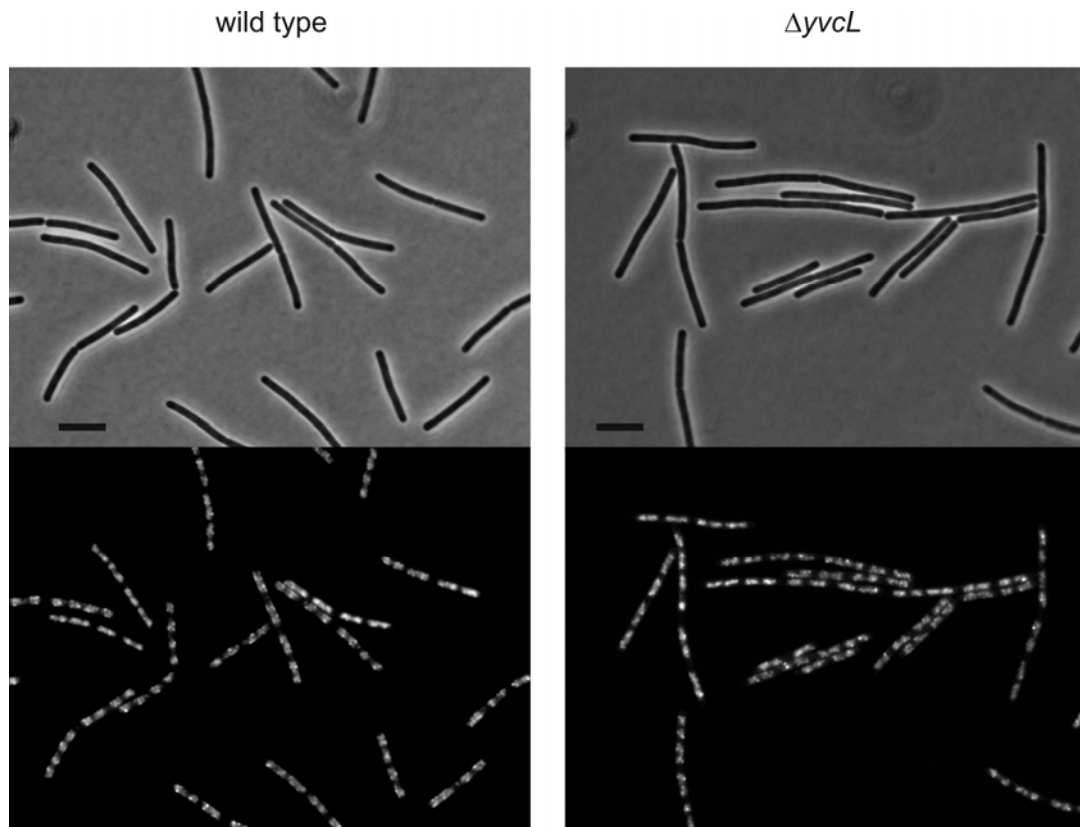


Fig. S5. Localization of Noc-YFP in *yvcL* mutant cells. For fluorescence microscopy, strains PG725 ( $P_{xyI}$ -*noc-yfp*) and PG727 ( $P_{xyI}$ -*noc-yfp*  $\Delta yvcL$ ) were grown in LB medium at 30°C, supplemented with 0.5% xylose to express Noc-YFP. Scale bar 5  $\mu\text{m}$ .

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