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Supplemental Information

Early Developmental Program Shapes Colony Morphology in Bacteria

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Figure S1

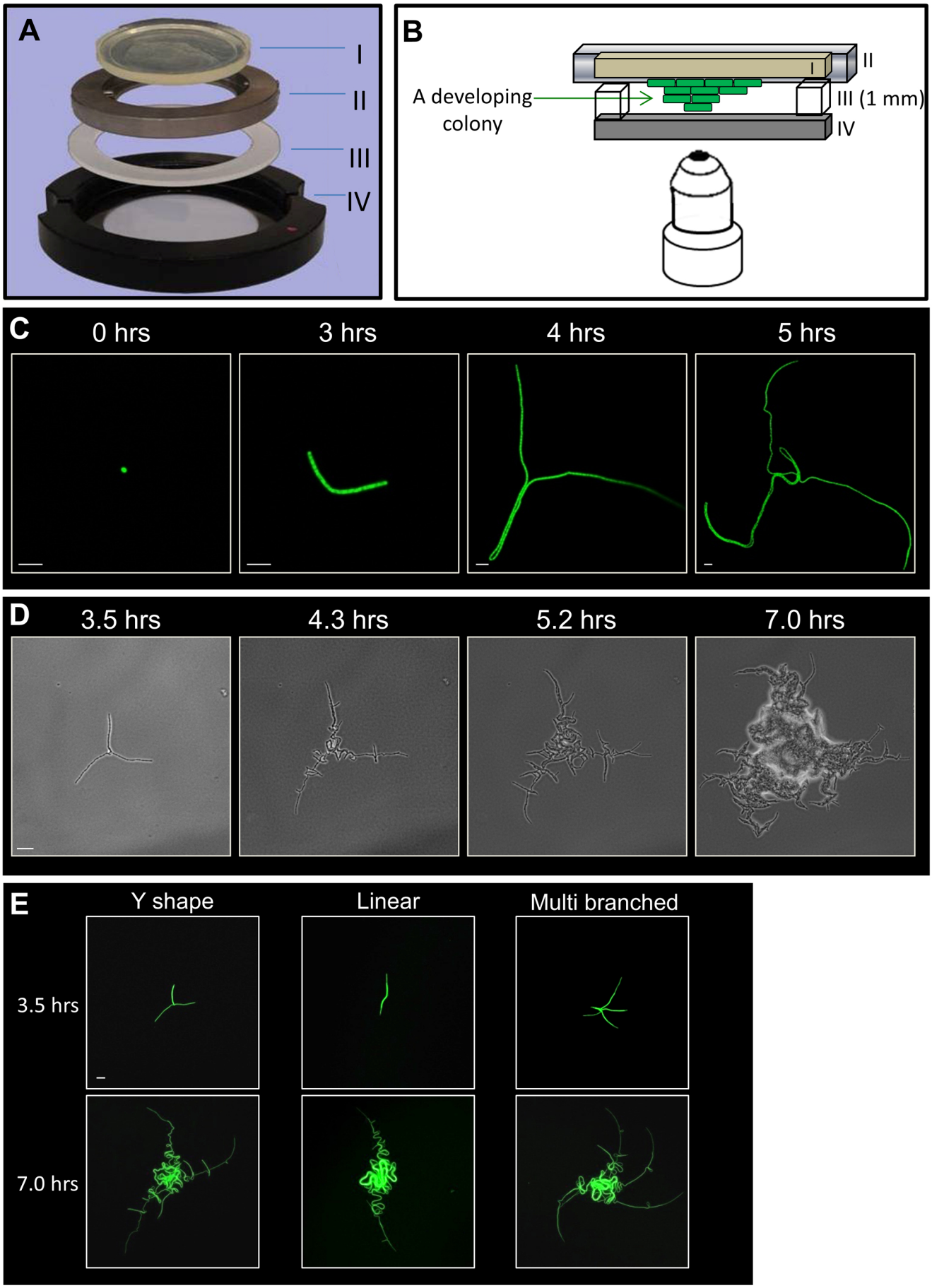
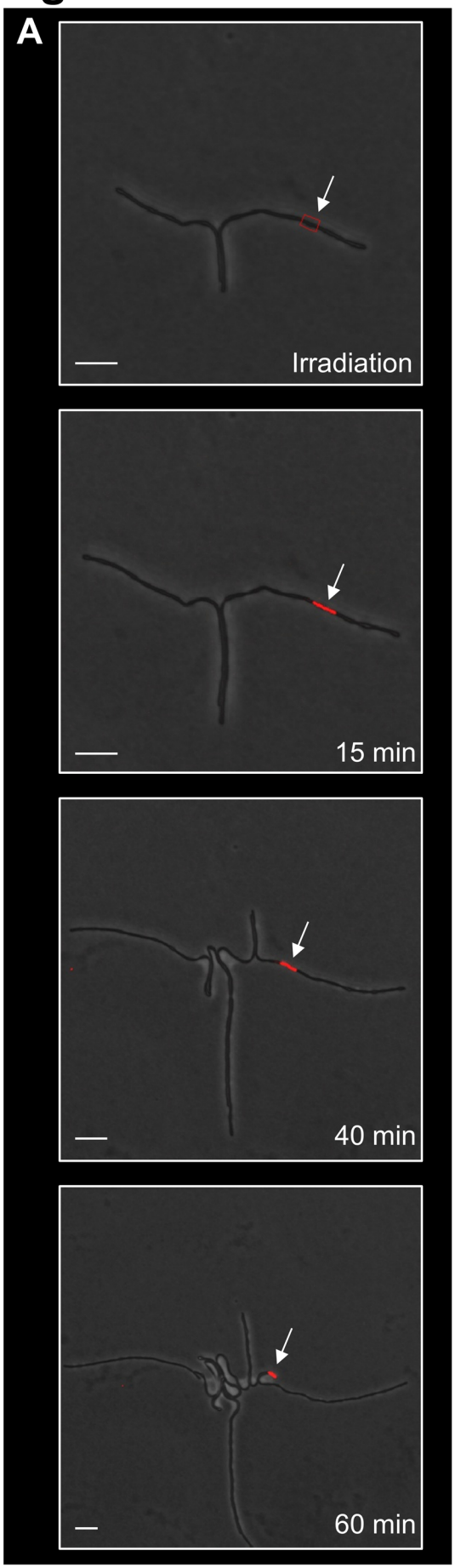

A
I
II
III
IV
B
A developing colony
II
III (1 mm)
IV
C
0 hrs
3 hrs
4 hrs
5 hrs
D
3.5 hrs
4.3 hrs
5.2 hrs
7.0 hrs
E
Y shape
Linear
Multi branched
3.5 hrs
7.0 hrs

Figure S2


A
Irradiation
15 min
40 min
60 min

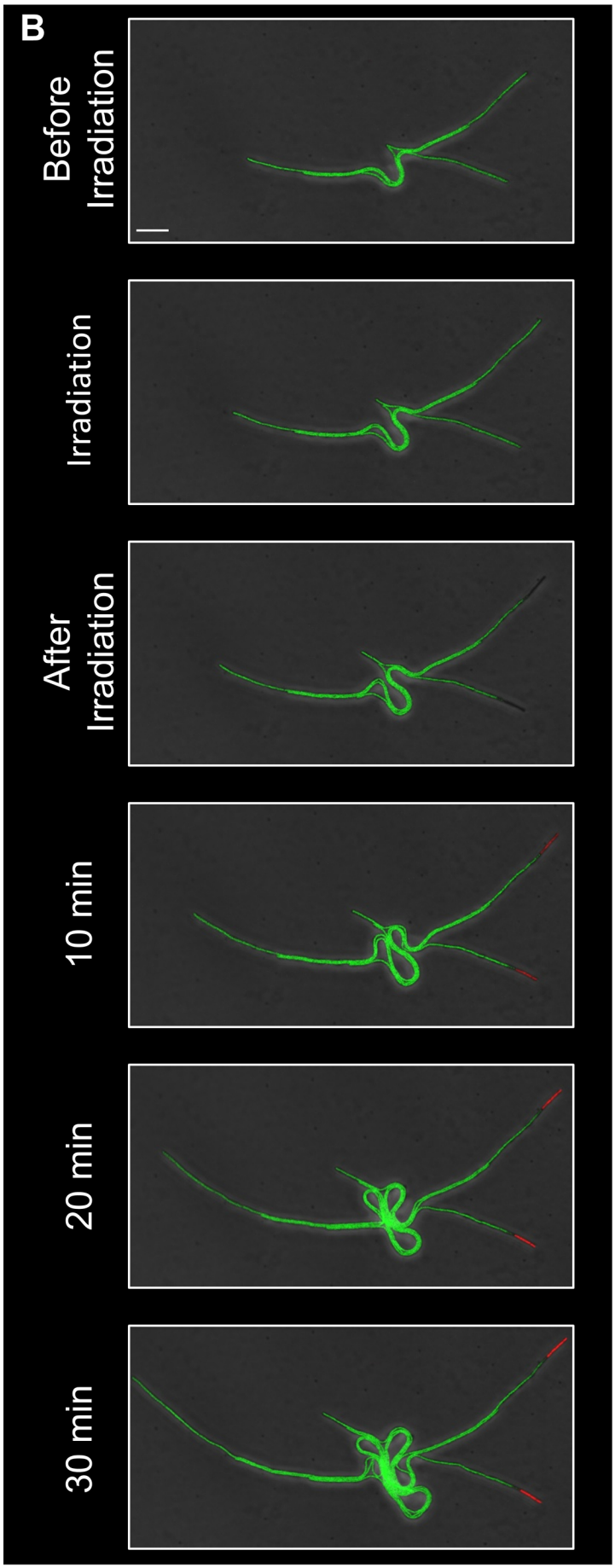
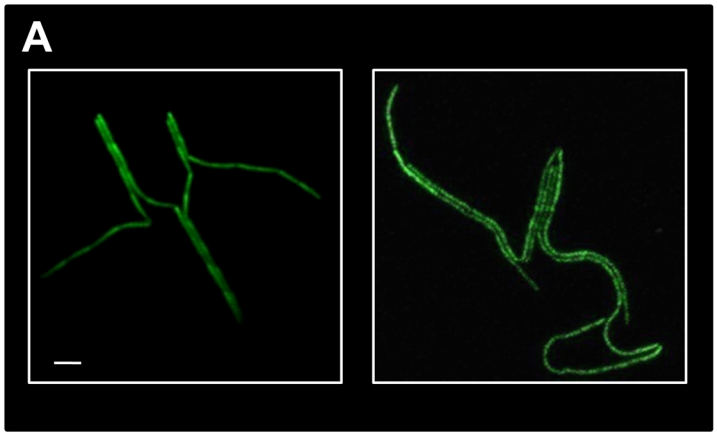
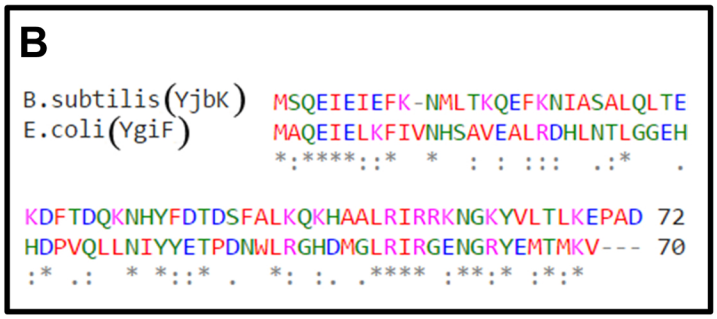
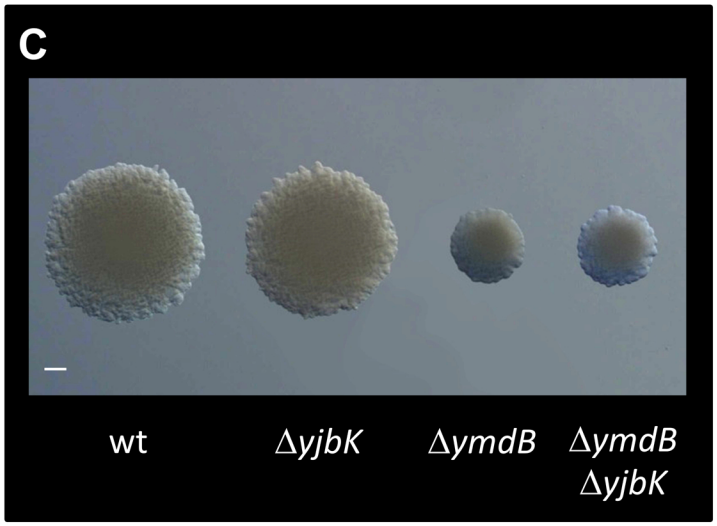
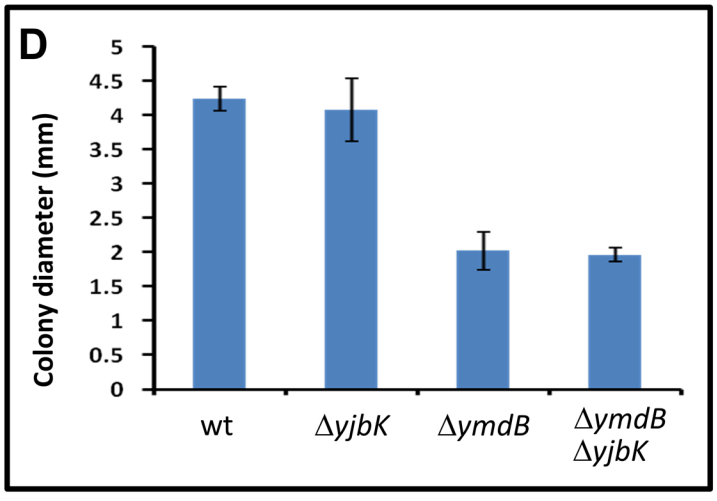

B
Before Irradiation
Irradiation
After Irradiation
10 min
20 min
30 min

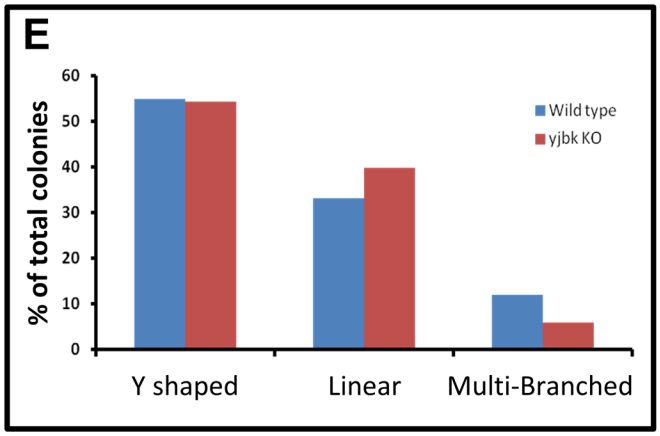
Figure S3

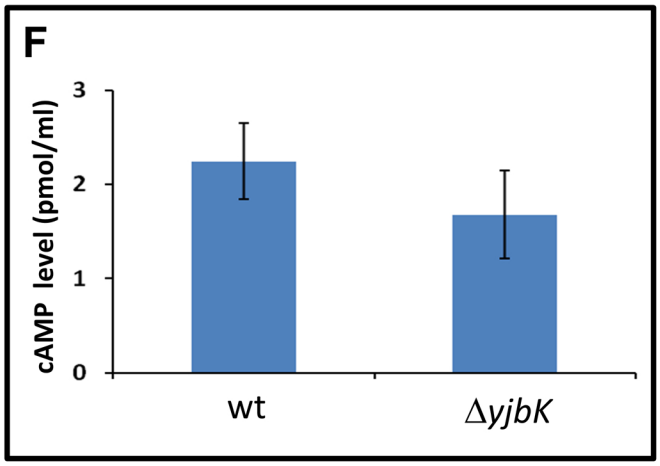

A


B
B.subtilis (YjbK) MSQEIEIEFK-NMLTKQEFKNIASALQLTE
E.coli (YgiF) MAQEIELKFIVNHSAVEALRDHLNTLGGEH
KDFTDQKNHYFDTDSFALKQKHAALRIRRKNGKYVLTLKEPAD 72
HDPVQLLNIYYETPDNWLRGHDMGLRIRGENGRYEMTMKV--- 70


C
wt
ΔyjbK
ΔymdB
ΔymdB ΔyjbK


D
Colony diameter (mm)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
wt
ΔyjbK
ΔymdB
ΔymdB ΔyjbK


E
% of total colonies
0
10
20
30
40
50
60
Wild type
yjbk KO
Y shaped
Linear
Multi-Branched


F
cAMP level (pmol/ml)
0
1
2
3
wt
ΔyjbK

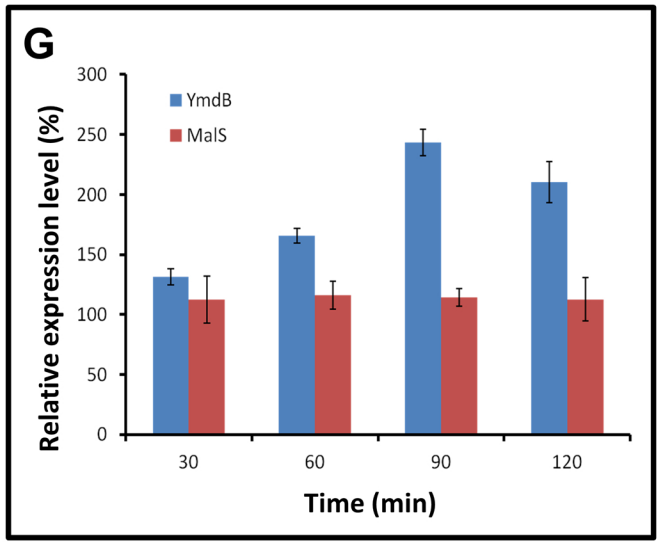

G
Relative expression level (%)
0
50
100
150
200
250
300
YmdB
MalS
30
60
90
120
Time (min)

Figure S4

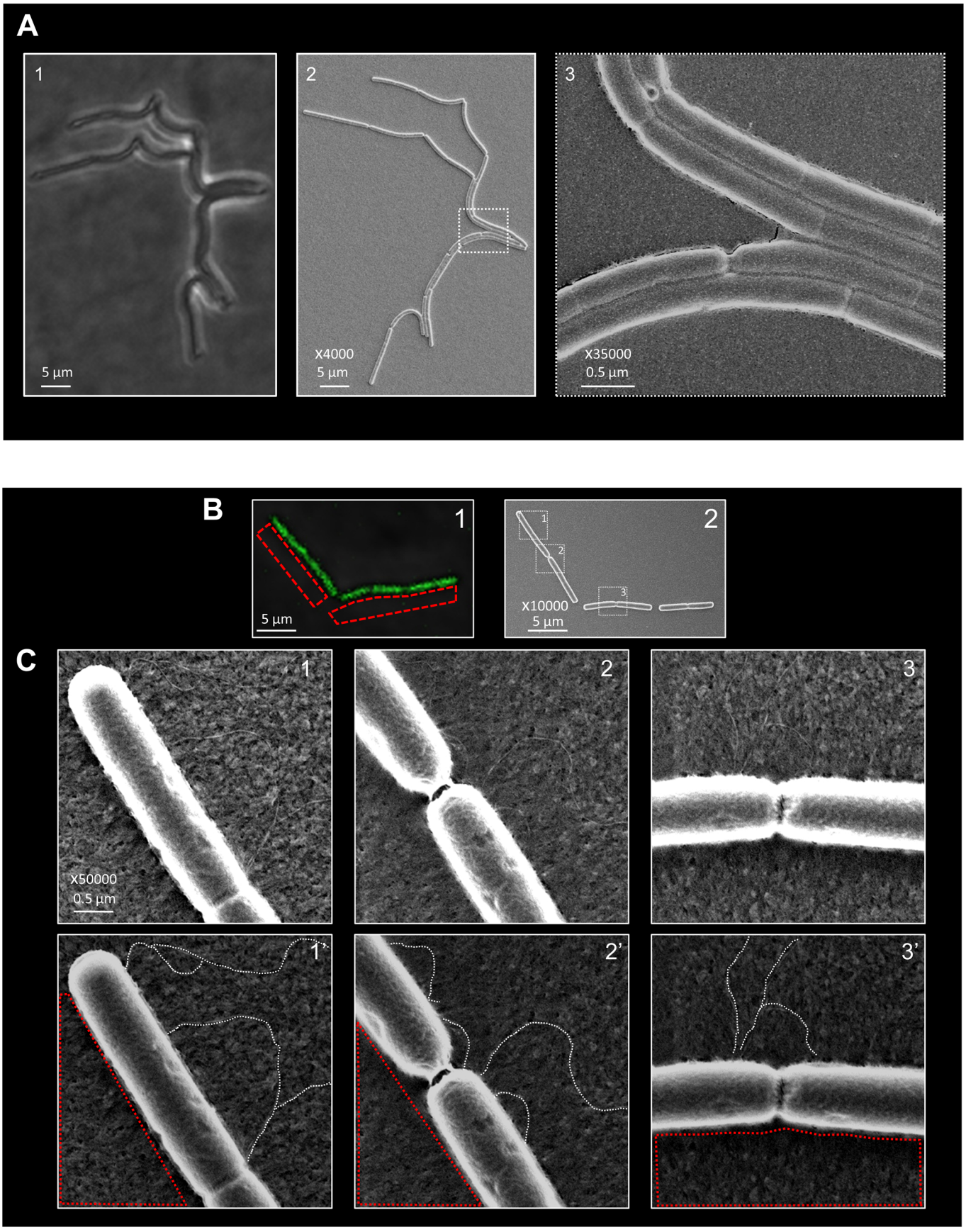
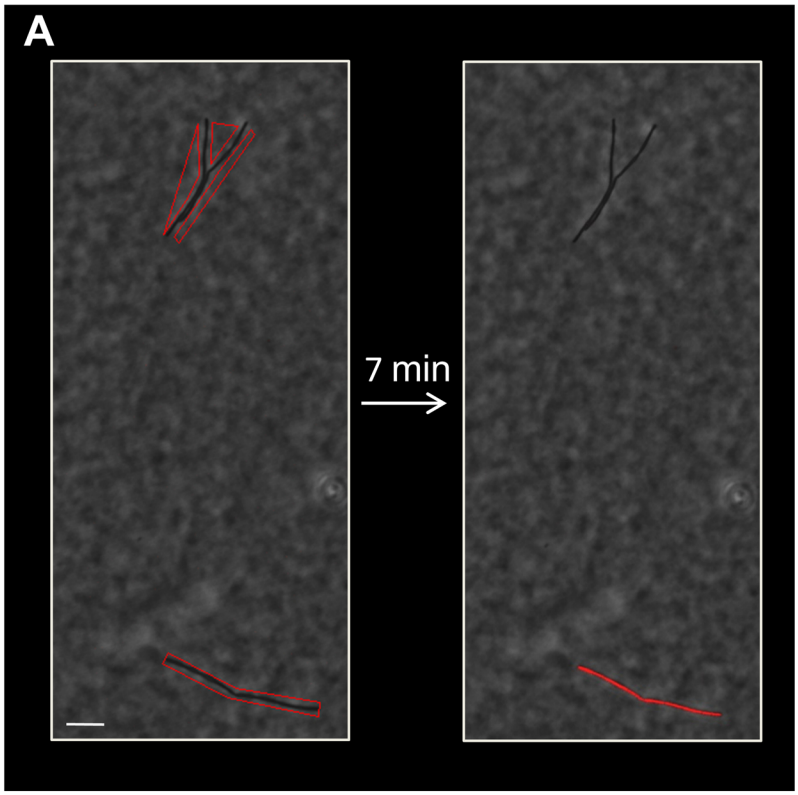
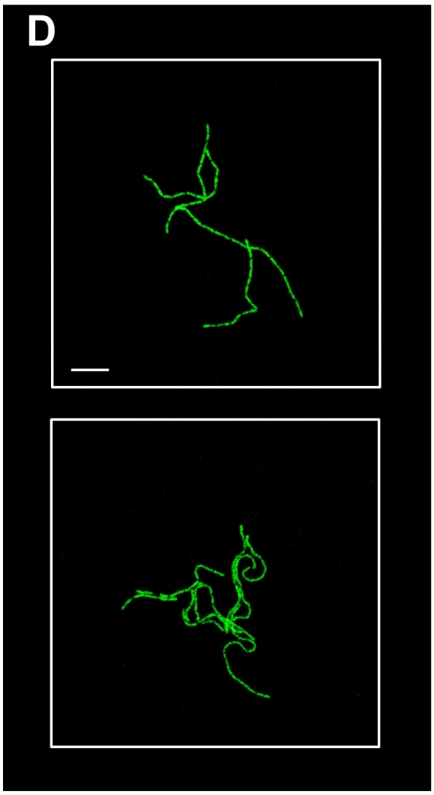
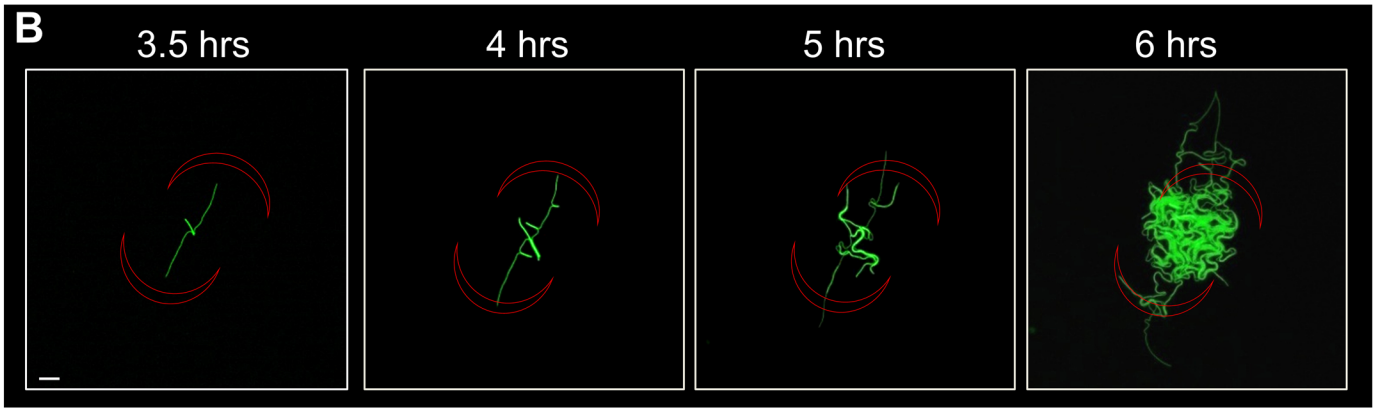

A
1
5 µm
2
X4000
5 µm
3
X35000
0.5 µm
B
1
5 µm
2
1
2
3
X10000
5 µm
C
1
X50000
0.5 µm
2
3
1'
2'
3'

Figure S5


A
7 min


D


B
3.5 hrs
4 hrs
5 hrs
6 hrs

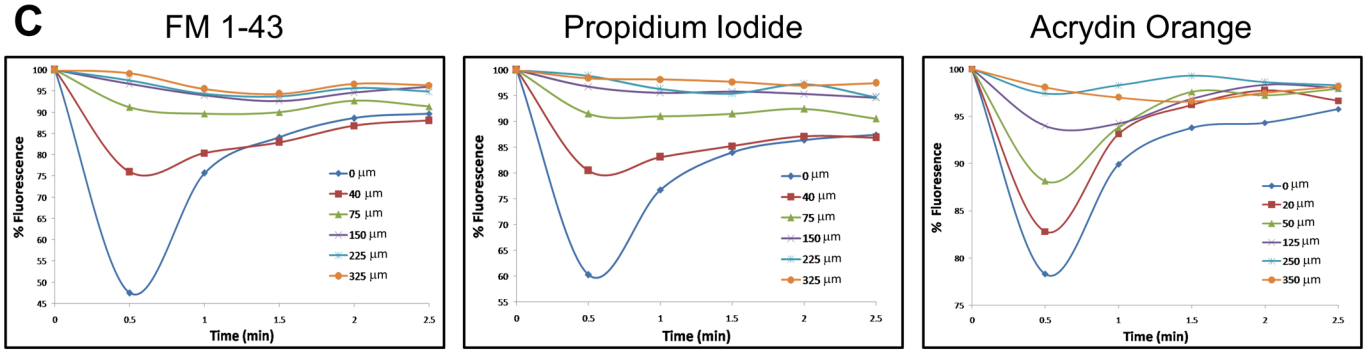

C
FM 1-43
Propidium Iodide
Acrydin Orange
% Fluorescence
Time (min)
0 µm
40 µm
75 µm
150 µm
225 µm
325 µm
20 µm
50 µm
125 µm
250 µm
350 µm

Table S1: List of genes examined in this study for the early stages of colony development

Strain name	Mutant gene(s)	Gene function	Observed effect on Y shape formation	Reference
GD215	Δ*hag*	Flagellum subunit	No effect	(Mirel and Chamberlin, 1989)
SG157	Δ*tasA*	Production of an ECM protein	No effect	(Branda et al., 2006; Serrano et al., 1999)
GD278	Δ*tasA*, Δ*hag*		No effect	
SB781	Δ*spo0A*	Master regulator of sporulation, affects biofilm formation.	Small effect, sometimes Y arms seem shorter	(Branda et al., 2001)
GD413	Δ*sinR*	Transcriptional regulator, repressor of many biofilm formation genes.	No effect	(Kearns et al., 2005)
GD420	Δ*sinI*	Antagonist of SinR	Small effect, sometimes Y arms seem shorter and curlier	(Kearns et al., 2005)
GM3	Δ*yvfA*	Antagonist of SinR	No effect	(Tortosa et al., 2000)
GM5	Δ*veg*	Antagonist of SinR	Small effect, sometimes Y arms seem shorter	(Lei et al., 2013)
GM9	Δ*ylbF*	Antagonist of SinR	No effect	(Branda et al., 2004)
GB61	Δ*ymdB*	Phosphodiesterase, affects biofilm formation	Major defect in Y shape formation	(Diethmaier et al., 2011)
GM7	Δ*ecsB*	ABC transporter, involved in multicellularity	No effect	(Branda et al., 2004)
GM8	Δ*yqeK*	Predicted hydrolase, involved in multicellularity	No effect	(Branda et al., 2004)
GM10	Δ*yhxB*	Alpha-phosphoglucomutase, involved in multicellularity	No effect	(Branda et al., 2004)

All the strains are derivatives of the wild type strain PY79
GB61 (Δ*ymdB*), having a profound effect, is highlighted in blue.

Table S2: List of bacterial strains used in this study

Strain Name	Genotype	Description
PY79	Wild type	(Youngman et al., 1984)
AR5	*rplA-gfp-spc*	(Rosenberg et al., 2012)
AR6	*rpsB-mCherry-kan*	Constructed by integrating pAR5 into PY79 (Segev et al., 2013).
AR16	*amyE::P_{rrnE}-gfp-spc*	(Rosenberg et al., 2012)
GB61	*Δymd B::tet*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *ymdB::tet*-P1-P4.
GB112	*Δymd B::tet, amyE::P_{rrnE}-gfp-spc*	Constructed by transforming AR16 gDNA into GB61.
GB113	*ymdB-gfp-spc*	Constructed by integrating pGB10 into PY79.
GB115	*Δymd B::tet, amyE::$P_{hyper\text{-}spank}$- ymdB-spc*	Constructed by transforming linearized pGB11 into GB61.
GB118	*Δymd B::tet, amyE::$P_{hyper\text{-}spank}$-ymdB-spc, rpsB-mCherry-kan*	Constructed by transforming AR6 gDNA into GB115.
GB125	*ymdB D8A*	PY79 was transformed with pGB16. Next, transformants were patched and screened for mls sensitivity, indicating that the plasmid was popped out. The presence of the mutation was confirmed by PCR followed by DNA sequencing.
GB142	*ΔyjbK::spc*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *yjbK::spc*-P1-P4.
GB154	*amyE::$P_{hyper\text{-}spank}$-yjbK-spc*	Constructed by transforming pGB37 into PY79.
GB156	*ΔyjbK::spc, Δymd B::tet*	Constructed by transforming GB61 gDNA into GB142.
GB201	*ymdB D8A-gfp-spc*	Constructed by transforming pGB10 into GB125.
GB222	*amyE::$P_{hyper\text{-}spank}$-yjbK-spc, ymdB-gfp -kan*	Constructed by transforming pGB13 into GB154.
GD215	*hag*::*erm*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers

		hag::erm-P1-P4.
GD278	*tasA::spec, hag::erm*	Constructed by transforming GD215 gDNA into SG157.
GD413	*sinR:spec*	Constructed using PCR based KO using primers *sinR::spec*-P1-P4.
GD420	*sinI:spec*	Constructed by transforming DS79 gDNA (a gift from D. Kearns, Indiana U) into PY79.
GM3	*yvfA:: pMUTIN -spec*	Genomic DNA from MGNA-B610 (*B. subtilis* Functional Analysis mutant collection) was inserted into PY79.
GM5	*Veg:: pMUTIN-spec*	Genomic DNA from MGNA-B909 (*B. subtilis* Functional Analysis mutant collection) was inserted into PY79.
GM7	*ΔecsB::spec*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *ecsB-ko* -P1-P4.
GM8	Δ*yqeK::kan*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers Δ*yqeK-ko*-P1-P4.
GM9	Δ*ylbF::tet*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *ylbF-ko*-P1-P4
GM10	Δ*yhxB::kan*	Constructed using Gibson assembly kit (NEB, USA) utilizing primers *yhxB-ko*-P1-P4.
GM13	*ΔyjbK, amyE::*$P_{hyper\text{-}spank}$*- yjbK-spc, malS- gfp -kan*	Genomic DNA from LS75 was inserted into GB154 strain
LS75	*malS- gfp -kan*	Constructed by integrating pLS75 into PY79.
SB781	*spoA::erm*	Constructed by transforming PY79 with SIK31(Ireton et al., 1993).
SG157	*tasA::spec*	Constructed by transforming gDNA from strain bDR2181 (a gift from David Rudner, Harvard U) into PY79.
NCIB 3610	Undomesticated *B. subtilis* strain	(Branda et al., 2001)
Bacillus cereus	Natural isolate, 16S rDNA sequencing defined it as TA32-5 strain	*B. cereus* 16S rDNA sequencing was conducted using primers 7F and 1511R.

Table S3: List of plasmids used in this study

Plasmid	Genotype	Description
pAR5	*rpsB-mCherry-kan*	(Segev et al., 2013)
pGB10	*ymdB-gfp-spc*	Constructed by amplifying the *ymdB* from gDNA of wild type *B. subtilis* strain (PY79), using primers *ymdB*-U-*EcoR*I and *ymdB*-L-*Xho*I. The PCR-amplified DNA was digested with *EcoR*I and *Xho*I and was cloned into pKL147 (Lemon and Grossman, 1998) digested with the same enzymes.
pGB11	*amyE::*$P_{hyper\text{-}spank}$*-ymdB-spc*	Constructed by amplifying the *ymdB* from gDNA of *B. subtilis* strain (PY79), using primers *ymdB*-U-*Hind*III and *ymdB*-L-*Sph*I. The PCR-amplified DNA was digested with *Hind*III and *Sph*I and was cloned into pDR111 digested with the same enzymes. pDR111 is a gift from D. Rudner (Harvard U).
pGB13	*ymdB-gfp-kan*	Constructed by amplifying the 3' region of *ymdB* from genomic DNA of wild type *B. subtilis* strain (PY79), using primers *ymdB*-U-*EcoR*I and *ymdB*-L-*Xho*I. The PCR-amplified DNA was digested with *EcoR*I and *Xho*I and was cloned into pKL168 digested with the same enzymes.
pGB15	*ymdB-erm*	Constructed by amplifying the *ymdB* ORF genomic DNA of wild type *B subtilis* strain (PY79), using primers *ymdB-SDM*-U-*BamH*I and *ymdB-SDM*-L-*Hind*III. The PCR-amplified DNA was digested with *BamH*I and *Hind*III and was cloned into pMAD digested with the same enzymes.
pGB16	*ymdBD8A-erm*	Constructed by amplifying the *ymdB* ORF from pGB15, using primers *ymdB*-SDM8-U and *ymdB*-SDM8-L followed by *Dpn*I treatment for 1 hr to digest the methylated DNA template. Insert was sequenced to confirm the presence of the mutation.
pGB37	*amyE*::$P_{hyper\text{-}spank}$*-yjbK-spec*	Constructed by amplifying the *yjbK* ORF from genomic DNA of wild type *B. subtilis* strain (PY79), using primers *yjbK* -U-*Hind*III and *yjbK*-L-*Sph*I. The PCR-amplified DNA was digested with *Hind*III and *Sph*I and was cloned into pDR111 digested with the same enzymes.
pLS75	*malS-gfp-kan*	Constructed by amplifying the 3' region of *malS* from genomic DNA of wild type *B. subtilis* strain (PY79), using primers *malS*-U-*EcoR*I and *malS*-L-*Xho*I. The PCR-amplified DNA was digested with *EcoR*I and *Xho*I and was cloned into pKL168 digested with the same enzymes.

Table S4: List of primers used in this study

Primer Name	**Primer Sequence (5'-3')**
ecsB-ko –P1	TCCAGAACCCTGTCTCTTTATGACAG
ecsB-ko –P2	ACATGTATTCACGAACGAAAATCGAGGCCAGCGTCTTCCTTTGTAA
ecsB-ko –P3	ATTTTAGAAAACAATAAACCCTTGCAAAGCTGTGAACTGAAAAGAGGTAATC
ecsB-ko –P4	GGCGAGGATTTTGCTTATTACTTACA
hag- ko –P1	GAAAATACAATATACTCCGTCACAGC
hag- ko –P2	ATTATGTCTTTTGCGCAGTCGGCTGTTTTGTTCCTCCCTGAATATGTTG
hag- ko –P3	CATTCAATTTTGAGGGTTGCCAGTAATTTTAAAAAAGACCTTGGCGTTG
hag- ko –P4	CTGTTGTTTCGCCAGGCGCAAC
yqeK-ko –P1	TCTTTCCAATTGTGGACTGGACA
yqeK -ko –P2	ATCACCTCAAATGGTTCGCTGGGTTTCCATTCTCCTCTACATACTTCTTCACTT
yqeK -ko –P3	AAGTTCGCTAGATAGGGGTCCCGAGCAACAGGAGGAATTTGTGAATGAACC
yqeK -ko –P4	GTTCTTTGCTCGTGTGTCTCATC
ylbF-ko –P1	CAGATAAGGCCGTTTCCCATC
ylbF-ko –P2	GAACAACCTGCACCATTGCAAGAAACATGCACCTCCAGTTGTTAG
ylbF-ko –P3	TTGATCCTTTTTTTATAACAGGAATTCAGACTGGCCGTCTCGTCTTTTAC
ylbF-ko –P4	CGAATATACAATGCTCAAACCCGCG
yhxB-ko-P1	TGGGAACAACAGACACTGTTTAC
yhxB-ko-P2	ATCACCTCAAATGGTTCGCTGGGTTTCATATTCGTCCTCCTATGTATCAGC
yhxB-ko-P3	AAGTTCGCTAGATAGGGGTCCCGAGCTTCAAGTATGAGCTGGGTCATTG
yhxB-ko-P4	GCGCACCATAGCTTTTAATGG

yjbK::spc:-P1	CTTTTCTGGCAAAAAGCATCTCTTTTACG
yjbK::spc:-P2	CTGAGCGAGGGAGCAGAAGATGCTCTTCCTTTCCGCCCTGTAAATC
yjbK::spc:-P3	GTTGACCAGTGCTCCCTGTAAGGTGGAATGACATCGTGAATGTAAC
yjbK::spc:-P4	CTGACAGCCGGGGTTTGTATTTC
ymdB::tet-P1	GAGATGAAAGGACGTATCATCGGAC
ymdB::tet-P2	GAACAACCTGCACCATTGCAAGATTTGTAAATCCTTTCTTCTTGAAAATTCC
ymdB::tet-P3	TTGATCCTTTTTTTATAACAGGAATTCTAGTTGAACATATGGTTATTTTATAAAAATATTAAAAAG
ymdB::tet-P4	TGCTGACATTCTCTCCCACGCCCTC
ymdB-U-*EcoR*I	AAACCCGAATTCACATATGTGAAAGCAAACGGCAAAG
ymdB-L-*Xho*I	ACCTAGCTCGAGTTCAAGAACATGTGATCATCGTTG
ymdB-U-*HinD*III	AAACCCAAGCTTAAAGGTGGTGAACTACTATGAGAATTTTATTTATCGGAGATGTTGTC
ymdB-L-*Sph*I	ACCTAGGCATGCTTATTATTATTCAAGAACATGTGATCATCGTTG
ymdB-SDM-U-*BamH*I	AAACCCGGATCCGTAGAAGGAAGCCACGTTGAGATCGGGG
ymdB -SDM-L-*Hind*III	TAGTTTAAGCTTCTAAGGAGTTGCTATATTCCTGCTTTTC
ymdB-SDM8-U	GAATTTTATTTATCGGAGCTGTTGTCGGTTCACCGGG
ymdB-SDM8-L	CCCGGTGAACCGACAACAGCTCCGATAAATAAAATTC
yjbK -U-*Hind*III	GATCGAATTCCATGCTGACCAAACAAGAATTCAAAAAC
yjbK-L-*Sph*I	CCCAAACTCGAGTATAGACTTTCGTTTTTCTTCATAAAAACGC
malS-U-*EcoR*I	TGGACTGAATTCCCGCCAGTTGAATATAACGGAGTTAC
malS-L-*Xho*I	TGGACTCTCGAGTATCGCGCGAATCGGTTTGTATA

Supplemental Figure Legends

Figure S1, Related to Figure 1. Initiation morphologies of *B. subtilis* developing colonies

(**A**) A chamber allowing the visualization of a developing colony by CLSM. (I) Solid medium allowing bacterial growth. The developing colony grows over the bottom side of the solid surface. (II) A metal ring holding the solid medium. (III) A 1 mm thick silicon ring. The ring creates the 3D space between the media and the cover slip, essential for colony growth. (IV) A holding apparatus harboring a cover slip. The apparatus holds the components together and fits a standard Lab-Tek insert.

(**B**) An illustration of a cross section of the assembled components in A as well as the position of the growing colony and the microscope lens.

(**C**) *B. subtilis* spores expressing *rplA-gfp* (AR5) were diluted, plated on LB solid medium and incubated at 37°C. Fluorescence images were taken by CLSM at the indicated time points. Scale bars 5 μm.

(**D**) Cells of the undomesticated *B. subtilis* strain NCIB 3610 were diluted, plated on LB solid medium and incubated at 37°C. Transmitted light images were taken by CLSM at the indicated time points. Colony development was generally similar to the lab strain PY79, though the Y arms of 3610 developing colonies tend to branch more frequently. Scale bar 20 μm.

(**E**) Cells expressing P_{rrnE}-*gfp* (AR16) were diluted, plated on LB solid medium, incubated at 37°C, and colony formation was followed by CLSM. Shown are the three typical morphologies of developing colonies at stage I (t=3.5 hrs; upper panels) and at stage III (t=7 hrs; lower panels): Y shape- having three arms; Linear- having two arms; Multi branched- having at least 4 arms. Scale bar 10 μm.

Figure S2, Related to Figure 2. Tracking Y arm extension

(**A**) Cells (PY79) were diluted, plated on LB solid medium, incubated at 37°C, and colony formation was followed by CLSM. At the stage of Y shape formation, cells were irradiated at the indicated position of the single chained Y arm (red frame, arrow). The irradiated cells were marked by the addition of PI (red signal), and their location (highlighted by arrows) was tracked at the indicated time points. Shown are overlaid transmitted light and fluorescence images. Scale bars 20 μm.

(**B**) Cells expressing P_{rrnE}-*gfp* (AR16) were grown as described in A. At the stage of Y shape formation, cells at the tip of 2 single chained Y arms were irradiated, and the irradiated cells

were marked by the addition of PI (red signal). The calculated length of the non irradiated arm at 30 min was extended by 71%, while the length of the two irradiated arms increased by ~16% during that time. Shown are representative experiments out of 13 independent biological repeats. Scale bar 20 μm.

Figure S3, Related to Figure 4. YjbK is a potential adenylate cyclase in *B. subtilis*

(**A**) *ymdB-D8A-gfp* (GB201) cells were diluted, plated on LB solid medium and incubated at 37°C. Fluorescence images of two colonies at t=3.5 hrs, time corresponding to Y shape formation, exemplifying the aberrant morphologies. Scale bar 5 μm.

(**B**) Sequence alignment of the *B. subtilis* YjbK N-terminal region compared with the *E. coli* predicted adenylate cyclase YgiF, showing a clear homology of the CYTH domain. Based on database searches using PSI-BLAST and on reference (Iyer and Aravind, 2002).

(**C**) PY79 (wt), Δ*yjbK* (GB142), Δ*ymdB* (GB61) and Δ*ymdB* Δ*yjbK* (GB156), were grown on solid LB medium and incubated at 37°C for 20 hrs. Shown are typical colonies photographed using a binocular. Scale bar 0.5 mm.

(**D**) The average diameter of 20 colonies described in C.

(**E**) Distribution of each of the initial colony morphology patterns as described in Figure 1 for wild type (PY79) and Δ*yjbK* (GB142) strains. For each strain at least 100 colonies were scored.

(**F**) Intracellular cAMP level of 1 ml cells (OD_{600} 0.7 ~ $5x10^8$ cells/ml) of wild type (PY79) and Δ*yjbK* (GB142) strains grown in liquid medium as measured by ELISA assay.

(**G**) $P_{hyper\text{-}spank}$-*yjbK*, *ymdB-gfp* (GB222) cells were diluted, plated on LB solid medium with or without IPTG, incubated at 37°C and followed by time lapse microscopy. Fluorescence images were captured at the indicated time points post IPTG induction, and the GFP level of cells grown with or without IPTG was measured. Shown are the relative expression levels of YmdB-GFP with IPTG/ YmdB-GFP without IPTG (blue bars). As a control, a similar experiment was conducted with $P_{hyper\text{-}spank}$-*yjbK*, *malS- gfp* (GM13), expressing the unrelated GFP fusion to the metabolic enzyme MalS. MalS-GFP expression was not affected by over expressing YjbK. For each time point at least 50 cells were scored.

Figure S4, Related to Figure 5. Perturbing the formation of nanotube networks

(**A**) Δ*ymdB* (GB61) cells were diluted, plated on a thin layer of LB solid medium, incubated at 37°C and colony formation was followed by CLSM. At t=3.5 hrs cells were fixed, washed,

gold coated and observed with HR-SEM. A GB61 developing colony, exemplifying the abnormal morphology of the mutant colony. (1) Phase contrast image. (2) HR-SEM image of the same colony. (3) Magnification of the boxed region in 2.

(**B**) Cells expressing P_{rrnE}-*gfp* (AR16) were diluted, plated on LB solid medium, incubated at 37°C, and colony formation was followed by CLSM. At t=2 hrs the marked regions (red frames) were irradiated using 405 nm laser beam at full power (B_1). Next, the cells were fixed, washed, gold coated and observed with HR-SEM (B_2).

(**C**) High magnification images of the boxed regions in B_2 (1-3), and their interpretive cartoons illustrating nanotubes positions (white lines) and the irradiated area (red lines) (1'-3'). A clear difference in nanotube abundance can be seen between the irradiated and non-irradiated regions of the same cells.

Figure S5, Related to Figure 5. Testing the effect of irradiation and SDS addition on colony development

(**A**) Cells (PY79) were diluted, plated on LB solid medium containing PI, incubated at 37°C and colony formation was followed by CLSM. The red framed regions (cells or their surrounding area) were irradiated using 405 nm laser beam (left) and photographed 7 min later (right). Shown are overlaid transmitted light and fluorescence images. PI (red signal, right) was able to penetrate only the cells that were directly irradiated. Furthermore, cells that their surrounding area was irradiated continued to grow, indicating that they remained intact. Scale bar 10 μm.

(**B**) Intercellular regions distant from the center of a developing colony residing in stage I were irradiated using 405 nm laser beam (red frames). Shown are fluorescence images of a colony taken at the indicated time points. Red frames in t=4,5,6 hrs highlight the area irradiated at t=3.5 hrs. Scale bar 20 μm.

(**C**) Fluorescent dyes (FM1-43, PI, Acrydin Orange) were added to LB agar and a marked region (30 μm^2) was bleached using 405 nm laser beam. Shown are FRAP charts of the 3 different fluorescent stains from areas located at the indicated distances from the center of the irradiated region (0 μm). The quick recovery of the fluorescent signal indicates that small molecules are rapidly diffused through the agar.

(**D**) Cells expressing P_{rrnE}-*gfp* (AR16) were diluted, plated on LB solid medium containing 0.007% SDS, incubated at 37°C, and colony formation was followed by CLSM. Shown are

two representative examples of developing colonies at t=3.5 hrs of development. Scale bar 10 μm.

Supplemental Movie Legends

Movie S1, Related to Figure 1. Y shape formation

Time lapse images of Y shape formation. Cells expressing P_{rrnE}-*gfp* (AR16) were diluted, plated on LB solid medium and incubated at 37°C in a 2D construct. Fluorescence images were taken by CLSM using 100X immersion lens at 12 min intervals in the course of 2 hrs.

Movie S2, Related to Figure 2. *B. subtilis* colony development

Time lapse images of colony development. Cells expressing P_{rrnE}-*gfp* (AR16) were diluted, plated on LB solid medium and incubated at 37°C. Fluorescence images were taken by CLSM at 15 min intervals in the course of 8 hrs.

Movie S3, Related to Figure 3. Irradiating one arm leads to asymmetric colony growth

Time lapse images of colony development following Y arm eradication. Cells expressing P_{rrnE}-*gfp* (AR16) were diluted, plated on LB solid medium and incubated at 37°C. At t= 3 hrs one arm of the developing colony was irradiated using 405 nm laser. Fluorescence images were taken by CLSM at 20 min intervals in the course of 6 hrs.

Movie S4, Related to Figure 5. *B. subtilis* colony development on SDS containing medium

Time lapse images of colony development. Cells expressing P_{rrnE}-*gfp* (AR16) were diluted, plated on LB solid medium supplemented with 0.007% SDS and incubated at 37°C. Fluorescence images were taken by CLSM at 20 min intervals in the course of 3.5 hrs.

Movie S5, Related to Figure 2. *B. cereus* colony development

Time lapse images of *B. cereus* colony development. *B. cereus* cells were diluted, plated on LB solid medium and incubated at 37°C. Transmitted light microscopy images were taken by CLSM at 20 min intervals in the course of 6 hrs.

Supplemental Experimental Procedures

General Methods

All general methods were carried out as described previously (Harwood and Cutting, 1990). For colony development experiments, growth was carried out at 37°C. Cells were incubated in liquid Luria Broth (LB) medium at 23°C overnight, and diluted to cell density of approximately 1 cell/μl (accordingly, a typical start up culture of OD_{600} 0.7 was diluted up to 10^{-5}). Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, when indicated. Sodium dodecyl sulfate (SDS) was added to a final concentration of 0.007% when indicated. Mature colonies grown overnight on LB plates were observed and photographed using Discovery V20 steroscope (Zeiss) equipped with Infinity1 camera (Luminera). Sporulation was induced by suspending cells in Schaeffer's liquid medium (DSM) (Schaeffer et al., 1965).

Measuring the Radial Area Occupied by a Developing Colony

Calculating the radial area occupied by a given colony was carried out by measuring the area of the minimal circle harboring the entire population of cells at the analyzed stage. The measured area was then normalized to the total number of cells in the developing colony, as estimated by measuring the total chain length. For *ymdB* mutant cells the area of at least 12 developing colonies was averaged and compared with the averaged area of 12 wild type colonies grown side by side.

Testing Diffusion Rates of Small Molecules on Agarose Pads

LB agarose pad (1.5%) was supplemented with the fluorescent dyes FM 1-43 (3 μg/ml) (Invitrogene), PI (20 μg/ml) (Sigma) or Acrydin Orange (40 μg/ml) (Sigma). A circled region (30 μm^2) was bleached at the center of the field using 405 nm laser at full power (5 mW, 75 iterations). Fluorescence levels from areas surrounding the bleached region at different distances were monitored every 0.5 min for 2.5 min.

cAMP ELISA Test

Enzyme-Linked Immunosorbent Assay (ELISA) test was conducted using Direct cAMP ELISA kit (ENZO Life Sciences). Cells were grown in liquid LB at 23°C over night. For each tested strain, 4 ml of the liquid culture (OD_{600} 0.7, ~$5x10^8$ cells/ml) were centrifuged

and the liquid fraction was decanted. Cells were washed with PBSx1, centrifuged and suspended in 125 µl of 1.8 mg/ml lysozyme, and incubated at 37°C. After 30 min, 125 µl of 0.2 M HCl were added and samples were incubated at room temperature for 15 min. Next, 20 µl sample were taken for Bradford assay to normalize the total protein concentration, and 25 µl of 10% Triton X-100 were added to the remaining samples. Samples were incubated at room temperature for 20 min and then tested for uniformity of cell lysis with phase contrast microscopy. Next, samples were centrifuged at 800 rpm and the supernatant was taken for ELISA analysis according to the manufacturer protocol. In order to get high sensitivity of the assay the acetylated protocol was used. cAMP levels were calculated per 1 ml cell culture.

Supplemental References

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