In Bacillus subtilis LutR is part of the global complex regulatory network governing the adaptation to the transition from exponential growth to stationary phase

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Supplementary Figure Legends

Fig. S1. Regulatory regions of genes with no gel- shift observed. Electrophoretic Mobility Shift Assays were performed in the presence of putative regulatory regions of genes under the control of LutR and purified LutR-His_6 at the indicated concentrations. In each assay, 25 μl total reaction mixture supplemented with competitor DNA polydIdC (1 μg/μl), BSA (1 mg/ml). Promoter region of the unrelated gene *ywbH* was used as negative control. Positive and negative controls were run together with each EMSA employed. For detection, gels were treated with SYBR Green I Nucleic Acid Gel Stain (1/10.000, v/v) (Roche) and visualized with a UV transilluminator. Each gel-shift assay was repeated at least two times

Fig. S2. Putative LutR binding motif is displayed as sequence LOGO. Each stack represents a position in the sequence. The height of the individual letters in a stack is the probability of the letter at that position multiplied by the total information content of the stack

Fig. S3. Effect of *lutR* mutation on the expression of *spoIIE*. Cells were grown in DSM medium at 37^oC and *spollE*-directed β-galactosidase activity was determined at the indicated times. Time zero denotes the end of exponential growth thus initiation of sporulation in DSM. β-galactosidase activity of *spoIIE::lacZ* fusion (squares) and its isogenic derivative *lutR* mutant (*spoIIE::lacZ::cat*, *lutR::Tn10::spc*) (triangle). Error bars indicate the standard deviation of the mean of three independent experiments (n=3).

Supplementary Table:

Table S1: Genes that are up (A)- and down (B)- regulated by LutR during early-stationary phase (OD_{600}7)

A.

Gene	Microarray[¶]	qPCR[‡]	EMSA[#]	Function	Transcriptional Organisation[§]
aprX	1.57	2.98(±0.17)	-	serine protease	≠ aprX ymaC ymaD ≠ ebrB
atpI	1.19	0.56(±0.03)	+	ATP synthase subunit I	
atpB	1.93			ATP synthase subunit A	
atpE	2.02	1.53(±0.15)		ATP synthase subunit C	≠ atpC atpD atpG atpA atpH
atpF	1.99			ATP synthase subunit B	atpF atpE atpB atpI ≠
atpH	1.93			ATP synthase subunit delta	
atpA	2.01	1.13(±0.10)		ATP synthase subunit alpha	
atpG	1.91			ATP synthase subunit gamma	
bacA[†]	ND		+	bacilysin biosynthesis protein, dehidratase	
bacB	ND			isomerase component of bacilysin synthetase	≠ ywfA ≠ bacA bacB bacC bacD
*bacC**	NR			bacilysin biosynthesis oxidoreductase	bacE ywfG ≠ ywfH ≠ ywfI
bacD	ND			alanine-anticapsin ligase	
*bacE**	NR			efflux protein for bacilysin excretion, self-protection against bacilysin	
*ywfG**	NR			transaminase	
ywfH[†]	ND		+	carrier protein reductase of bacilysin biosynthesis	bacE ywfG ≠ ywfH ≠ ywfI
bioW	1.99	1.46(±0.06)	-	6-carboxyhexanoate--CoA ligase	
bioA	1.38			adenosylmethionine--8-amino-7-oxononanoate transaminase	bioI ≠ bioB bioD bioF bioA bioW
bioF	1.45			8-amino-7-oxononanoate synthase	≠ ytaP msmR
bioD	2.06			dithiobiotin synthetase	
bioB	0.36			biotin synthase	
bslA	3.33	2.70(±0.25)	+	hypothetical protein	yuaC ≠ bslA ≠ yuaA yubG ≠
cwlO	2.24	1.70(±0.05)	+	secreted cell wall DL-endopeptidase	yvcI trxB cwlO ≠ yvcD yvcC ≠
*czcD**	NR	2.25(±0.22)	+	potassium/proton-divalent cation antiporter	yrdR yrdQ ≠ trkA czcD ≠ yrdN ≠
*trkA**	1.76	1.02(±0.06)	-	potassium uptake oxidoreductase	
epsD	2.21	2.63(±0.05)	-	extracellular matrix biosynthesis enzyme	
epsE	2.08	1.34(±0.31)		Glycosyltransferase	≠ sigL yvfG epsO epsN epsM
epsK	1.49	1.10(±0.21)	-	extracellular matrix component exporter	epsL epsK epsJ epsI epsH epsG
epsN	0.79	1.02(±0.01)		aminotransferase	epsF epsE epsD epsC epsB epsA
epsO	1.79	0.94(±0.05)		pyruvyl transferase	slr
fabHA	1.87	1.00(±0.12)	-	3-oxoacyl-(acyl carrier protein) synthase III	

fabF	1.89			3-oxoacyl-(acyl carrier protein) synthase II	*comZ yjzB fabHA fabF ≠yjaZ*
fabHB	3.00	1.98(±0.05)	-	3-oxoacyl-(acyl carrier protein) synthase III	*yhgE≠fabHB yhfC ≠yhfD yhfE*
fapR	1.36	1.16(±0.09)	-	fatty acid biosynthesis transcriptional factor	*recG fapR plsX fabD fabG*
plsX	1.66			putative glycerol-3-phosphate acyltransferase PlsX	
fabD	2.42		-	malonyl CoA-acyl carrier protein transacylase	*ylpC plsX fabD fabG acpA ≠ rnc ≠smc ≠ftsY*
fabG	2.28			beta-ketoacyl-acyl carrier protein reductase	
acpA	2.29		-	acyl carrier protein	*fabG acpA ≠rnc ≠smc*
ftsE	1.11	1.94(±0.13)	+	cell-division ABC transporter (ATP-binding protein)	*yvjB ≠ftsX ftsE ≠cccB yvjA≠*
ftsX	1.68			cell-division ABC transporter	
gtaC	1.52	2.04(±0.06)	-	integral inner membrane protein	*≠galT galK gtaC ywcC ywcB*
hepS	1.35	0.91(±0.18)	+	Heptaprenyl diphosphate synthase component I	*≠ndk hepT menH hepS mtrB mtrA ≠hbs ≠spoIVA≠*
hepT	1.52			Heptaprenyl diphosphate synthase component II	
ndk	1.37	1.01(±0.24)		nucleoside diphosphate kinase (purine nucleotide biosynthesis)	
lip	2.38	2.65(±0.09)	+	secreted alkaliphilic lipase	*≠lmrB lmrA yccC ≠lip ≠yczC ≠*
*murE**	ND	2.02(±0.05)	+	UDP-N-acetylmuramoyl-L-alanyl-D-glutamyl-meso-2,6-diaminopimelate synthetase	*≠ spoVD murE mraY murD ≠ spoVE*
mraY	1.45	1.20(±0.06)		phospho-N-acetylmuramoyl-pentapeptide-transferase	
murD	1.53			UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	
pksD	1.9	1.44(±0.06)	-	enzyme involved in polyketide synthesis	*pksA ≠ pksB pksC pksD pksE acpK pksF ≠pksG pksH pksI pksJ pksL pksM pksN pksR ≠ pksS ymzB*
pksE	1.59			enzyme involved in polyketide synthesis	
acpK	1.77			acyl-carrier protein	
pksF	ND			malonyl-ACP decarboxylase	
pksG	2.10	1.42(±0.18)	-	acetyl-S-AcpK beta-ketothioester polyketide intermediate transferase	
pksH	0.42			enzyme involved in polyketide synthesis	
*pksI**	0.36			enzyme involved in polyketide synthesis	
pksJ	2.73	4.14(±0.01)		polyketide synthase of type I	
pksL	3.95	6.19(±0.06)		polyketide synthase of type I	
pksM	2.17			polyketide synthase	
pksN	2.96			polyketide synthase of type I	
pksR	2.46			polyketide synthase	
*ppsA**	NR		+	plipastatin synthetase	*≠ppsE ppsD ppsC ppsB ppsA ≠*
ppsB	1.1	1.09(±0.18)		plipastatin synthetase	
*ppsC**	NR			plipastatin synthetase	
ppsD	1.24			plipastatin synthetase	
ppsE	2.42			plipastatin synthetase	

pyrR	2.52	2.79(±0.32)	+	bifunctional pyrimidine regulatory protein PyrR uracil phosphoribosyltransferase	≠ $\overline{pyrR}$ ≠ $\overline{pyrP}$ ≠$\overline{pyrB}\,\overline{pyrC}$
*pyrP**	NR	3.48(±0.18)		uracil permease	
pyrB	1.37	4.34(±0.01)	+	aspartate carbamoyltransferase catalytic subunit	≠ $\overline{pyrR}$ ≠ $\overline{pyrP}$ ≠ $\overline{pyrB}$ $\overline{pyrC}$ $\overline{pyrAA}$ $\overline{pyrAB}$ $\overline{pyrK}$ $\overline{pyrD}$ $\overline{pyrF}$ $\overline{pyrE}$ ≠$\overline{cysH}$
pyrC	1.89			Dihydroorotase	
pyrAA	2.31	5.85(±0.20)		carbamoyl-phosphate synthetase (glutaminase subunit)	
pyrAB	2.00			carbamoyl-phosphate synthetase (catalytic subunit)	
pyrK	0.85			dihdrooratate dehydrogenase	
pyrD	1.32			dihdrooratate dehydrogenase (catalytic subunit)	
pyrF	1.21			orotidine 5'-phosphate decarboxylase	
pyrE	1.08			orotate phosphoribosyl transferase	
rnc	2.41	1.64(±0.03)	-	ribonuclease III, cleaves both 5'- and 3'-sites of the small cytoplasmic RNA precursor	$\overline{acpA}$ ≠$\overline{rnc}$ ≠$\overline{smc}$ ≠$\overline{ftsY}$ ≠$\overline{ylqB}$
smc	1.42			chromosome condensation and segregation SMC ATPase	
ftsY	1.08			signal recognition particle	
rplJ	1.69	3.42(±0.30)		50S ribosomal protein L10	$\overline{nusG}$ $\overline{rplK}$ $\overline{rplA}$ ≠ $\overline{rplJ}$ $\overline{rplL}$ ≠ $\overline{ybxB}$
rplL	1.66			50S ribosomal protein L7/L12	
sdpA	1.26	1.08(±0.22)	+	sporulation delay protein-export of killing factor	$\overline{yvaV}$ $\overline{sdpA}$ $\overline{sdpB}$ $\overline{sdpC}$ ≠
sdpB	2.2	1.68(±0.08)		Sporulation delay protein-exporter of killing factor SpbC	
sdpC	0.61			killing factor SdpC	
spoIIE†	ND		+	serine phosphatease	≠$\overline{spoIIE}$ $\overline{yabS}$ $\overline{yabT}$ $\overline{yacA}$ $\overline{hprT}$
tapA	0.96	2.65(±0.06)	+	lipoprotein for biofilm formation	$\overline{sinI}$ $\overline{sinR}$ ≠≠ $\overline{tasA}$ $\overline{sipW}$ $\overline{tapA}$ $\overline{yqzG}$ $\overline{yqzE}$
sipW	2.12	1.42(±0.14)		type I signal peptidase	
tasA	1.50	2.65(±0.17)		major biofilm matrix component	
tig	1.68	1.11(±0.01)	-	trigger factor (prolyl isomerase), catalyze in vitro protein folding; essential for growth under starvation conditions	≠$\overline{lonA}$ ≠$\overline{lonB}$ $\overline{clpX}$ $\overline{tig}$ $\overline{ysoA}$ ≠
tsf	1.69	1.31(±0.53)	-	Ts elongation factor	$\overline{sigD}$ $\overline{ylxL}$ $\overline{rpsB}$ ≠$\overline{tsf}$ $\overline{pyrH}$ $\overline{frr}$ ≠
pyrH	1.52	1.74(±0.15)		uridylate kinase	
frr	1.55	1.11(±0.08)		ribosome recycling factor	
ydjM	2.33		+	hypothetical protein	≠$\overline{ydjL}$ $\overline{ydjM}$ ≠$\overline{ydjN}$ ≠$\overline{ydjO}$
ydjN	1.48			hypothetical protein	
yhdN	ND		-	aldo/keto reductase	$\overline{yhdN}$ ≠$\overline{plsC}$ ≠$\overline{yhdP}$ $\overline{yhdQ}$ $\overline{yhdR}$ ≠
plsC	1.49	1.05(±0.14)	-	1-acyl-sn-glycerol-3-phosphate acyltransferase (lipid metabolism)	
yhfE	1.63	3.65(±0.04)	+	putative endoglucanase	≠$\overline{fabHB}$ $\overline{yhfC}$ ≠$\overline{yhfD}$ $\overline{yhfE}$ $\overline{yhfF}$ ≠
yhfF	1.40	2.18(±0.09)		hypothetical protein	
yjcM	1.48			hypothetical protein	≠$\overline{yjcK}$ $\overline{yjcL}$ ≠$\overline{yjcM}$ $\overline{yjcN}$ ≠$\overline{yjcO}$
ylxM	1.32		-	hypothetical, DNA-binding protein	

ffh	1.51	2.91(±0.04)	-	signal recognition particle-like (SRP) GTPase	*rnc* ≠ *smc ftsY* ≠ *ylqBylxM ffh* ≠
yokD	NR	2.65(±0.25)	+	aminoglycosideN3'-acetyltransferase	≠ *yokF yokE* ≠ *yokD yokC*
yqgA	1.80			hypothetical protein	*yqgC yqgB* ≠ *yqgA yqfZ yqfY* ≠
yqxI	1.31			hypothetical protein	≠ *yqzI yqcG yqcF* ≠ *yqxJ yqxI*
yqxJ	1.78			hypothetical protein	
yukE	1.46	1.29(±0.06)	+	hypothetical protein	≠ *yueD yueC yueB yukA yukB yukC yukD yukE yukF* ≠
yukD	0.85			Putative bacteriocin	
yukC	1.75			hypothetical protein	
yukB	0.46			hypothetical protein	
yukA	1.48			hypothetical protein	
yueB	0.6			Bacteriophage SPP1 adsorption protein	
*yvcA**	NR	1.56(±0.01)	+	Lipoprotein	*yvcC* ≠ *yvzA yvcB yvcA* ≠ *hisI*
yybN	1.76	2.05(±0.24)	+	hypothetical protein	*yybN* ≠ *yybM yybL yybK yybJ yybI yybH yybG* ≠ *yybF*
yybM	0.87			integral inner membrane protein	
yybL	1.52	1.23(±0.12)	-	integral inner membrane protein	
yybK	1.73			integral inner membrane protein	
yybJ	1.52			ATP-binding cassette protein	
yydF	ND		+	biactive peptide eliciting cell envelope stress sensed by the LiaRS TCS	≠ *yydJ yydI yydH yydG yydF fbp*
yydG	1.93	1.68(±0.09)		putative AdoMet radical enzyme	
yydH	0.62			membrane-embedded protease	
yydI	0.51			YydIJ: an ATP-binding cassette (ABC) transporter	
yydJ	0.73				

B.

Gene	Microarray[¶]	qPCR[‡]	EMSA[#]	Function	Transcriptional Organisation[§]
*abh**	NR	-4.57(±0.28)	-	transcriptional regulator of transition state genes (AbrB-like)	≠ *ykpC mreBH abh* ≠ *kinC ykqA*
acoA	-1.57	-3.07(±0.22)	+	acetoin dehydrogenase E1 component (TPP-dependent alpha subunit)	≠ *yfjM yfjL* ≠ *acoA acoB acoC acoL* ≠ *acoR sspH* ≠
acoB	-1.54			acetoin dehydrogenase E1 component (TPP-dependent beta subunit)	
acoC	-1.56			branched-chain alpha-keto acid dehydrogenase subunit E2	
acoL	-1.36			dihydrolipoamide dehydrogenase	
aprE	-2.35	-3.07(±0.91)	+	extracellular alkaline serine protease (subtilisin E)	≠ *yhfM yhfN* ≠ *aprE yhfO yhfP* ≠
argC	-1.63	-1.98(±0.22)	-	N-acetyl-gamma-glutamyl-phosphate reductase	*yitY yitZ* ≠ *argC argJ argB argD carA carB argF* ≠ *yjzC*
argJ	-1.89			bifunctional ornithine acetyltransferase/N-acetylglutamate synthase protein	
argB	-2.08			acetylglutamate kinase	
argD	-2.13			acetylornithine aminotransferase	

carA	-2.22			carbamoyl phosphate synthase small subunit	
carB	-2.15			carbamoyl phosphate synthase large subunit	
argF	-2.16			ornithine carbamoyltransferase	
argG	-1.68		+	argininosuccinate synthase	≠ *ytzD* *argH* ≠ *argG* ≠ *moaB* ≠ *ackA* ≠
argH	-2.04			argininosuccinate lyase	
bceA	ND	-2.68(±0.32)	+	bacitracin ABC efflux transporter ATP-binding protein	≠ *yttB* *yttA* ≠ *bceB* *bceA* *ytsB* *ytsA* ≠ *ytrF*
bceB	ND	-3.77(±0.06)		bacitracin export permease protein BceB	
citA	-1.50	-2.20(±0.30)	-	citrate synthase 1	≠ *citR* *citA* *yhdF* ≠ *yhdG* ≠ *yhdH* ≠
citB	-1.38	-2.21(±0.21)	-	aconitate hydratase	≠ *cotM* *sspP* *sspO* *citB* ≠ *yneN*
glnR	-1.31	-0.73(±0.10)	+	transcriptional regulator (nitrogen metabolism)	*spoVG* ≠ *ynbA* *ynbB* *glnR* *glnA* ≠
glnA	-1.19			glutamine synthase	
gltA	-1.31	-2.04(±0)	+	glutamate synthase (large subunit)	≠ *yofA* *yogA* ≠ *gltB* *gltA* *gltC* ≠ *proJ* *proH*
gltB	-1.45			Glutamate synthase [NADPH] small chain	
ispA	-2.01	-1.68(±0.22)	+	intracellular serine protease	≠ *metE* ≠ *ispA* *ykoB* ≠ *ykoC* *ykoD*
liaI	-1.65	-1(±0.05)	+	putative transmembrane protein	
liaH	-1.78			similar to phage-shock protein A(PspA) of *E. coli*	
liaG	-1.31			putative membrane-anchored hypothetical protein	*liaR* *liaS* *liaF* *liaG* *liaH* *liaI* ≠ *yvqJ* ≠ *yvqK*
liaF	-1.05			membrane protein	
liaS	-1.02			LiaRS: two component regulatory system	
liaR	-0.85				
msmR	-1.48	-1.43(±0.27)	+	transcriptional regulator (LacI family)	*bioW* ≠ *ytaP* *msmR* *msmE* *amyD* *amyC* *melA* ≠
msmE	-1.51			multiple sugar-binding lipoprotein	
pbpE	ND	-3.44(±1.13)	+	penicillin-binding protein 4* (spore cortex)	≠ *racX* *pbpE* *sacB* ≠ *yveB* ≠
racX	ND			amino acid racemase	
*rapI** [†]	-1.84(NR)		+	response regulator aspartate phosphatase	*yddK* *rapI* *phrI* ≠ *yddM* ≠ *yddN*
*phrI**	NR			phosphatase RapI regulator	
sigW	ND	-4.31(±0.01)	-	RNA polymerase sigma factor sigW	*trnSL-Gln2* ≠ *sigW* *ybbM* ≠ *ybbP*
ybbM	ND			hypothetical protein	
trpE	-1.9	-1.26(±0.05)	-	anthranilate synthase component I	
trpD	-2.1			anthranilate phosphoribosyltransferase	
trpC	-2.62			indole-3-glycerol-phosphate synthase	≠ *aroE* *tyrA* *hisC* *trpA* *trpB* *trpF* *trpC* *trpD* *trpE* *aroH*
trpF	-2.22			N-(5'-phosphoribosyl) anthranilate isomerase	
trpB	-2.00			tryptophan synthase subunit beta	
trpA	-1.04			tryptophan synthase subunit alpha	
ybfO	-1.76	-1.08(±0.06)	+	hypothetical protein; similar to erythromycin esterase	*psd* *ybfN* ≠ *ybfO* ≠ *ybfP* *ybfQ* ≠
ybyB	-1.48			hypothetical protein	≠ *ybxI* *cypC* ≠ *ybyB* *ybeC* ≠ *glpQ*
yceC	-1.00	-0.88(±0.06)	+	putative stress adaptation protein	

yceD	-1.44			putative stress adaptation protein	≠*ycdI* $\overline{yceA}$ ≠ $\overline{yceB}$ $\overline{yceC}$ $\overline{yceD}$ $\overline{yceE}$ $\overline{yceF}$ $\overline{yceG}$ $\overline{yceH}$ ≠$\overline{yceI}$
yceE	-1.39			putative stress adaptation protein	
yceF	-1.73	-1.38(±0.08)		putative stress adaptation transporter	
yceH	-1.43			putative stress adaptation protein	
yneN	-1.41	-1.04(±0.19)	+	putative membrane-bound protein with a thioredoxin-like domain	$\overline{sspO}$ $\overline{citB}$ ≠$\overline{yneN}$ $\overline{sspN}$ *tlp* ≠
yotH	-1.80			hypothetical protein	$\overline{yotI}$ $\overline{yotH}$ $\overline{yotG}$ $\overline{yotF}$ $\overline{yotE}$ $\overline{yotC}$
yuaF	-1.72	-1.12(±0.09)	+	membreane integrity integral inner membrane protein	$\overline{yuaJ}$ ≠ $\overline{yuaI}$ *floT* $\overline{yuaF}$ $\overline{yuaE}$ ≠ $\overline{yuaD}$ ≠
floT	-1.46			putative flotillin-like protein	
yuaI	-1.62			putative acetyl-transferase	

Genes in an operon are grouped together

¶ Numbers indicate the log 2 transformed expression ratio's.

‡ Numbers indicate the log 2 transformed expression ratio's. (The mean of minimum three independent replicate experiments is given and standart deviation of the mean is shown in paranthesis),

§ transcriptional organization retrieved from http://genolist.pasteur.fr/SubtiList/, "≠" indicates the termination sites

A (+) indicates that a gel retardation was observed

* Bayes P value higher than acceptable value (>0.01)

NR no reproducible data obtained

ND no differential expression observed

† Expression profiles were elucidated by *lacZ*-fusion analysis

Supplemantary Figures

Fig.S1

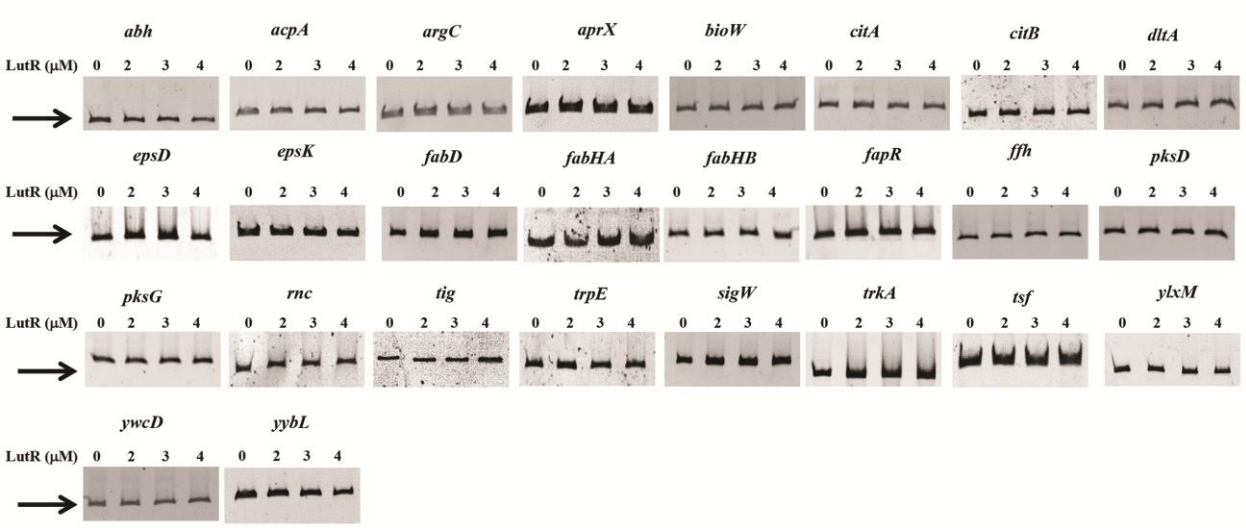


Fig.S2

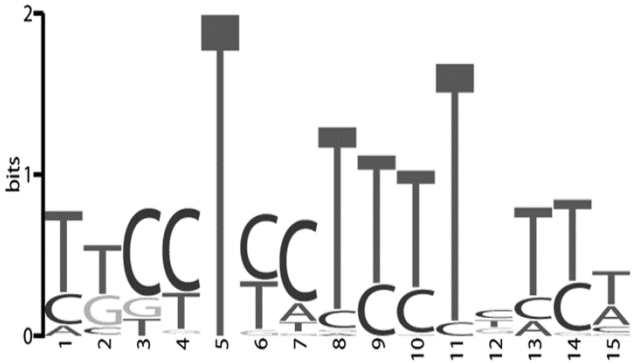
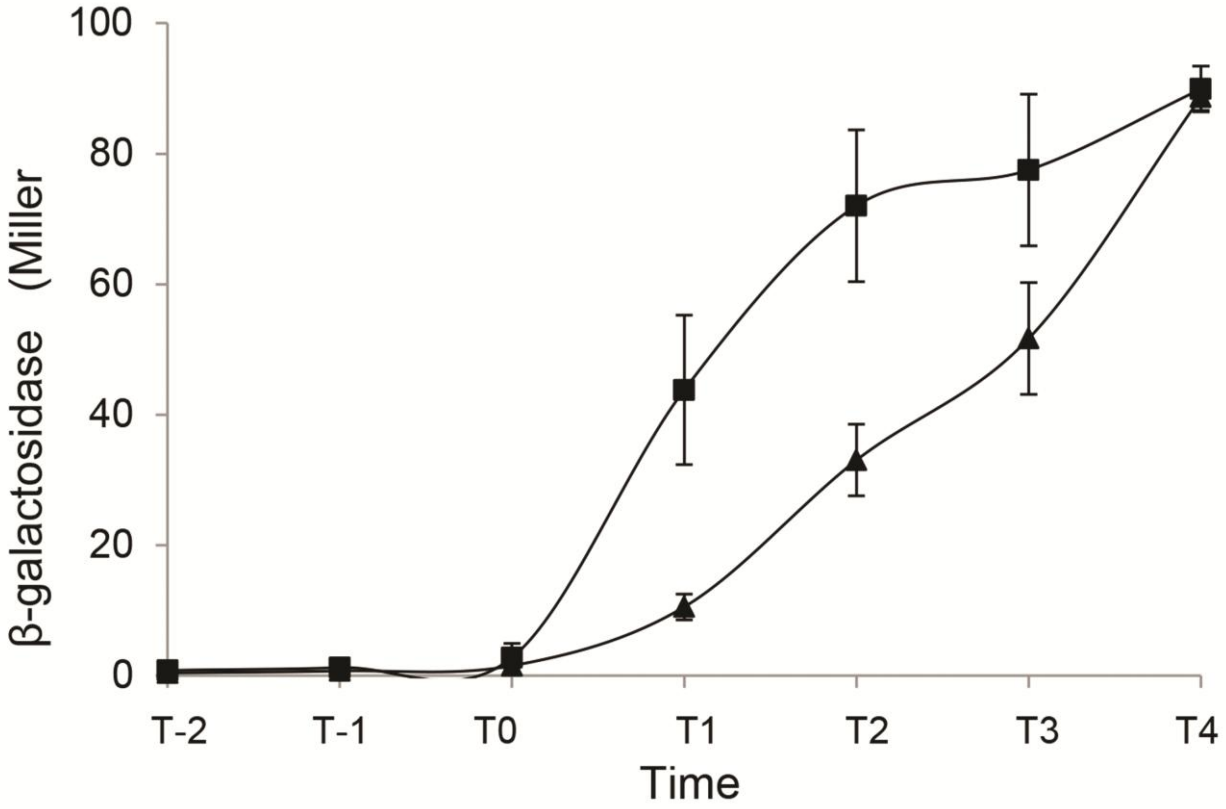


Fig.S3


100
80
60
40
20
0
β-galactosidase (Miller
T-2
T-1
T0
T1
T2
T3
T4
Time

Supplementary Methods:

Detailed information about the results of EMSA analysis performed in the presence of both LutR and SinR

It has been previously suggested that LutR and SinR seem to act cooperatively to repress *lutABC* (Chai *et al.,* 2009). To analyze the overlap between LutR and SinR DNA targets, we performed EMSA with the addition of either or both proteins to given promoters. As a first step, we analyzed the interaction in the case of the *lutA* promoter. As shown in Fig. 5, an apparent mobility shift was observed only at the highest LutR concentration tested (4 μM): neither 2 nor 3 μM LutR alone was able to produce a detectable mobility shift, but the presence of 3 μM SinR, which by itself had not shifted the DNA probe, greatly stimulated the binding capacity of LutR, almost all of the DNA probe was shifted even at 2 μM LutR. Consistently, only 4 μM SinR alone was able to produced a detectable shifted DNA complex, but its binding was stimulated by the presence of 3 μM LutR, almost all of DNA probe was shifted at 2 μM SinR, which itself had not shifted the DNA probe. On the other hand, neither LutR nor SinR alone, nor a mixture of them was able to shift the control DNA probe (the regulatory region of the unrelated *ywhB*), at all of the protein concentrations tested. These results elucidated not only the specific interactions of SinR and LutR with the P_{lutA} but also the cooperative nature of their interaction. Besides the *lutABC* operon, *tapA* operon and *aprE* gene were previously known to be under direct control of SinR and were found in our study to be directly regulated by LutR. Under light of these findings, we then wondered whether SinR would interact with the regulatory regions of all or only some LutR-target genes and it could stimulate the LutR binding capacity for those genes. For this, EMSA analysis described above were performed with the regulatory regions of all of the LutR-target genes identified in our study. Interestingly, as shown in Fig. 5, SinR was capable to interact with the regulatory regions of all of the LutR-target genes tested, but they exhibited variations in the nature of their interactions. Both LutR and SinR apparently stimulate each other's binding to the regulatory regions of *lip and bslA.* As an example, only 4 μM SinR alone could shift the regulatory regions of *lip,* but in the presence of 3 μM LutR which itself had not shifted the DNA probe, almost all of the DNA probe was shifted even at 2 μM SinR, which itself had not shifted the DNA probe SinR. Similarly, in the presence of 3 μM SinR, even 2 μM LutR, which itself had not shifted the DNA probe, shifted all of the DNA probe. Besides to cooperative interactions, they exhibited additive or simultaneous binding to the regulatory regions of *acoA, aprE, atpI, bacA, bceA, czcD, cwlO, ftsE, glnR, gltA, hepS, ispA, liaA, msmR, mraY, pbpE, ppsA, pyrB, pyrR, rapI, sdpA, tapA, ybfO, yceC, ydjM, yneN, yhfE, ywfH, yuaF, yukE, yvcA, yokD, yydF, yybN*. In these cases, both LutR and SinR alone was able to produce a mobility shift with the

regulatory regions. Addition of increased amount of LutR in the presence of fixed amount of SinR or addition of increased amount of SinR in the presence of fixed amount of LutR resulted in an additional retardation in the electrophoretic mobility of SinR-bound or LutR bound-DNA probes, suggesting a co-binding to these regions without promoting each other`s binding activity. Interestingly, in the case of the *spoIIE* gene, LutR apparently stimulated the binding capacity of SinR while SinR did not affect the binding capacity of LutR which itself had produced a mobility shift at all concentrations tested in a concentration dependent manner. SinR alone was not capable to produce a mobility shift at 2 or 3 μM protein concentrations even it produced at 4 μM concentration, but in the presence of 3 μM LutR, resulted in an additional retardation in the electrophoretic mobility of LutR bound-DNA probes. Specific inhibition of sporulation stage II genes: *spoIIA, spoIIE* and *spoIIG* by SinR had been reported previously (Mandic-Mulec *et al*., 1995) that provides an *in vivo* support for the *in vitro* interaction of SinR with the s*poIIE* regulatory region detected in this study.

Mandic-Mulec, I., Doukhan, L. & Smith, I. (1995). The *Bacillus subtilis* SinR protein is a repressor of the key sporulation gene *spo0A. J Bacteriol* **177,** 4619-4627.

Detailed information on the DNA-microarray analysis

Total RNA isolat ed from all samples using the Macaloid/Roche protocol (van Hijum *et al.*, 2005). 10-20 μg of total RNA was first reverse transcribed into cDNA with the Superscript III Reverse Transcriptase kit (Invitrogen) and labeled with a Cy3- or Cy5-monoreactive dye (GE Healthcare, Amersham). Purification of CyDye labeled cDNA was executed using NucleoSpin Extract II columns (Machery Nagel). Concentrations of Cy3 and Cy5 were expected to be at least 0.5 pmol/μl in a total volume of 50 μl. The labeled and purified cDNA samples were hybridized to oligonucleotide microarrays in Ambion Slidehyb #1 buffer (Ambion Europe Ltd) at 48 °C for more than 16 hours. The arrays were constructed as described elsewhere (van Hijum *et al.*, 2003a). Furthermore, slide spotting, slide treatment after spotting and also slide quality control were done as before (van Hijum *et al.*, 2005). Following hybridization, slides were washed for 5 min in 2x SSC with 0.5% SDS, 2 times 5 min in 1x SSC with 0.25% SDS, 5 min in 1x SSC 0.1% SDS for the removal of hybridization buffer and unbound cDNAs. Finally slides were dried by centrifugation (2 min at 2000 rpm) and scanning of the slides was performed in a GenePix 4200AL Microarray Scanner (Axon Instruments, CA, USA). Fluorescent signals were quantified using ArrayPro 4.5 (Media Cybernetics Inc., Silver Spring, MD). Following the acquisition of expression data,

expression levels were processed and normalized with Micro-Prep which is a helpful software tool that enables scientists to handle microarray data and transform raw data into processeable Excel data through avoidance of inconsistencies (van Hijum *et al.*, 2003b). Then the expression difference ratio of the *lutR* mutant and wild type PY79 strains during stationary growth were further processed using the Cyber-T tool, which is a software program that works on a t-test variant combined with a Bayesian statistical framework (Baldi & Long, 2001). Parameter use in the Cyber-T tool was followed as recommended, giving 2 as 'minimum non-zero replicates', a 'sliding window' of 101 and a confidence value of 10. Accepted Cyber-T (Bayes) *p* value was set to 0.01.

van Hijum, S. A., de Jong, A., Baerends, R. J., Karsens, H. A., Kramer NE, Larsen, R., den Hengst, C. D., Albers, C. J., Kok, J. & Kuipers, O. P. (2005). A generally applicable validation scheme for the assessment of factors involved in reproducibility and quality of DNA-microarray data. *BMC Genomics* **6**, 77.

van Hijum, S. A., de Jong, A., Buist, G., Kok, J. & Kuipers, O. P. (2003a). UniFrag and GenomePrimer: selection of primers for genome-wide production of unique amplicons. *Bioinformatics* **19**, 1580-1582.

van Hijum, S. A., Garcia de la Nava, J., Trelles, O., Kok, J. & Kuipers, O. P. (2003b). MicroPreP: a cDNA microarray data pre-processing framework. *Appl Bioinformatics* **2**, 241-244.

Primers used in this study

abh forward 5’ gat gaa tta ggc cgc att 3’

abh reverse 5’ ggc ttg aat ttc ttc gag 3’

abh EMSA forward 5’ gtc aat ccc gat ttc agt tga 3’

abh EMSA reverse 5’ gac aat gcg gcc taa ttc atc 3’

acoA EMSA.forward 5’ggc ggt att gga tat gtc aaa 3’

acoA EMSA.reverse 5’gta cat cca cag cgc ttt ttc 3’

acoA forward 5’ ctg gag atc agg ggc ttt 3’

acoE reverse 5’ aca gcc ttt ggc gat aca 3’

acpA EMSA forward 5’ cgg ttt aac caa atc ttc tgc 3'

acpA EMSA reverse 5’tac gat gat ttt cgt tac acg 3’

aprE EMSA forward 5’atc cat tgt tct cac gga agc 3’

aprE EMSA reverse 5’cgc aaa caa caa gct gat cca 3’

aprE Forward 5’ gcg ttc agc aac atg tct 3’

aprE Reverse 5’cac ata tgc aac gct cgg 3’

aprX EMSA forward 5’ gcc ggatcc ctg tct cac aat aat cgt tct 3’

aprX EMSA Reverse 5’ cgg gaa ttc cca atc tag ctt gtg agc att 3’

aprX F 5’ tgg act cct tgc ttc ttg 3’

aprX R 5’ tgc tga agg agt aac ctc 3’

argC EMSA forward 5’ggc ttt ctc gct gac ttt ttc 3’

argC EMSA reverse 5’tcc tgt agc acc tac aat tcc 3'

argC forward 5’ agc gag ggt tat cct cat 3’

argC reverse 5’ cag atc acc tga cag atc 3’

argG EMSA forward 5’caa tca tgt cga gag caa ctg 3’

argG EMSA reverse 5’acc tcc tga gta tgc taa tac 3’

argG forward 5’ gag ggc aaa gat ttg gca 3’

argG reverse 5’ acg aac ctg gtc att tcc 3’

atpA reverse 5’acc aac agg aac ctc cat 3’

atpA forward 5’gtc atc caa gtc ggt gac 3’

atpE reverse 5’gaa tgc gat aac gac agc

atpE forward 5’gca gct gcg att gca att 3’

atpI EMSA forward 5’- gcc ggatcc tca tgt gtc ttg tag cag cgc-3’

atpI EMSA reverse 5’- cgg gaattc cat tct tct gac gag cag taa-3’

atpI forward 5’ ttg gca gtg tat gta ctg ggt-3’

atpI reverse 5’ cgg gaa ttc cat tct tct gac gag cag taa-3’

bioW EMSA forward 5’aca ggg agc tgc tga ttg cta 3’

bioW EMSA reverse 5’cat tga agc cct cat tct gac 3’

bioW forward 5’gtc aga atg agg gct tca 3’

bioW reverse 5’ cac agg caa tgg ctg aat 3’

citA EMSA forward 5’gcc ttc atc agt cag ctg gat 3’

citA EMSA reverse 5’aca tgt aat tcc ctt taa tcc 3’

citA forward 5’ agc ttt gaa gaa gcg gct 3’

citA reverse 5’cgg atg gaa tgt gta cgt 3’

citB EMSA forward 5’cgt ttt caa tat agc cgg cgc 3’

citB EMSA reverse 5’cgc ttg gaa aac gtc ttt tga 3’

citB forward 5’ aag gtt tcg aag ctt cct 3’

citB reverse 5’ cag tga agc cag atc tac 3’

czcD forward 5’ tct gat gca ggc cat atg ctg 3’

czcD reverse 5’ aag cat gcc ggt tgt tgc tac 3’

czcD EMSA forward 5’ttg tag ttg aat act acc ctc 3’

czcD EMSA reverse 5’tgc tcc ttc att atg att gtg 3’

fabD EMSA forward 5’ctg gaa ggc tct gcg ttg tca 3’

fabD EMSA reverse 5’tga tcc ctg acc cgg gaa taa 3’

fabHA EMSA forward 5’cgg gct tga ttt gat caa gag 3’

fabHA EMSA reverse 5’acg tcc aac acc aag tat tcc 3’

fabHA forward 5’ gac gag tgg att cgt aca 3’

fabHA reverse 5’ gcc gag ttg ttc ttg aat 3’

fabHB EMSA forward 5’gca gta cat aaa cat gaa cgc 3’

fabHB EMSA reverse 5’ata ggt gcc gat agc tgt aat 3’

fabHB forward 5’ gaa tgg atc gtt cag cgc 3’

fabHB reverse 5’ gct ttc cca gcc gaa ata 3’

ffh EMSA forward 5’tat cag tcg ttg ttg acg tca 3’

ffh EMSA reverse 5’tcg gtc ggc taa tcc ttc aaa 3’

ffh forward 5’ atg atg cgt gag gtc cgt ctt-3’

ffh reverse 5’ aac ttt aat gac ctg ctg gcc-3’

frr forward 5’ tat tac gga gcg cag aca 3’

frr reverse 3’ccg tct ctc ttc tgt tag 3’

ftsE EMSA forward 5’taa aaa cgg ccg cgg caa tat 3’

ftsE EMSA reverse 5’gcc gtt cgg ata ggc ttt ata 3’

ftsE forward 5’ tat gtt gtt ggt ccg agc 3’

ftsE reverse 5’cac ttc aag ggc aaa tgc 3’

glnR forward 5’ cca gcc aga agt gaa gga 3’

glnR reverse 5’ tct cag ttc gtc atc gga 3’

glnR EMSA forward 5’tacatgcctggatacgaggat 3’

glnR EMSA reverse 5’tgg aaa taa agg cat tga gcg 3’

gltA EMSA forward 5’gat att tct ccc ttc acg ctc 3’

gltA EMSA reverse 5’acg gta gag acc ttg agc ttt 3’

gltA forward 5’ atc ggc cta tat gca cac 3’

gltA reverse 5’tcc tac ccc gta acg ttc 3’

hepS EMSA forward 5’gcc gtt gag gac gga gtg aat 3’

hepS EMSA reverse 5’gtt cag att ggc taa agt tcc 3’

hepS forward 5’ gcg aag cat att tct gcg 3’

hepS reverse 5’ gag aag agt caa ttg gcg 3’

ispA forward 5’ aag gcg cca gaa atg tgg 3’

ispA reverse 5’ agc tgc aat tgt tcc ggc 3’

ispA EMSA forward 5’act gtt gca tta tgt agg gcg 3’

ispA EMSA reverse 5’cac ata cgg gat caa gcg gat 3’

lip EMSA forward 5’aat ggt gtc gtc aca cca aac 3’

lip EMSA reverse 5’tac aag tgc aat gat cct tct 3’

lip forward 5’ gtc gtt atg gtt cac ggt 3’

lip reverse 5’ cat gct gtg agc gac aat 3’

murE.EMSA.forward 5’gat acg cgt gta ttt gac tga3’

murE.EMSA.reverse 5’ggt tgt taa gta tgt aag cag 3’

murE.forward 5’ gtc gat gtg aat gtt cct 3’

murE.reverse 5’ agg aag tgt ttc gtc tcc3’

mraY forward 5’gga ccg aaa tca cat cag 3’

mraY reverse 5’gcg ctt cat gac aac ctt 3’

msmR EMSA forward 5’aat gtc tca aca ctg att ggc 3’

msmR EMSA reverse 5’gga aac ttt agc ttt caa ggc 3’

msmR forward 5’ ctt tct gtt gcg ggc gaa 3’

msmR reverse 5’ ccc ttt ccg aat gga aga 3’

ndk forward 5’ gtc caa cgt cag ctc att 3’

ndk reverse 5’ cag ctg tct cgt cac ttc 3’

pbpE EMSA forward 5’gat tgc aac tgg tct att ttc 3’

pbpE EMSA reverse 3’taa cgt ctg aag atg ctt tct 3’

pbpE forward 5’- cag ttt aac ggg acg gtt-3’

pbpE reverse 5’-ctg ata cgg aaa acc ggg-3’

pksD EMSA forward 5’aac cat cgc tcc cac atc tag 3’

pksD EMSA reverse 5’ata gta ttg gga acc ttg ccc 3’

pksD forward 5’ cga atc ggc aca tcc att 3’

pksD reverse 5’ cca gac tga tcc caa tac 3’

pksG EMSA forward 5’gaa tta aag gcg ctc cgt gca 3’

pksG EMSA reverse 5’att cat cgc ttc tat tcc ggc 3’

pksG forward 5’ ctt gat gtc atg gag ctg 3’

pksG reverse 5’ gtt gcg gtt gag acc taa 3’

pksJ forward 5’ ccg gct cca tta gcc gtt 3’

pksJ reverse 5’ tcc cgt act gcc tga agt 3’

pksL EMSA forward 5’agc gcc gct ttg tcc cat ttt 3’

pksL EMSA reverse 5’ctt ttt cac gtt aga cct cca 3’

pksL forward 5’ aag gct gac atg cac gca 3’

pksL reverse 5’ caa atg att ggc acc cac 3’

ppsA EMSA forward 5’caa tta gaa gaa tga tgc aca 3’

ppsA EMSA reverse 5’ttg ggc atg ggt taa aga ata 3’

ppsB forward 5’ aat cta tgc gtc gac tcg 3’

ppsB reverse 5’agc atc aat cag cgt ctg 3’

pyrAA forward 5’tct tac tgc gga cag atc 3’

pyrAA reverse 5’ atc aat tcc ctg gag tcc 3’

pyrB EMSA forward 5’cag tta tcc ttg tca tcg gtg 3’

pyrB EMSA reverse 5’agt gct aag ttc act cat cgt 3’

pyrB forward 5’ttc gaa ccg agc acg aga 3’

pyrB reverse 5’ ctg gct gac aag ctc ttc 3’

pyrH forward 5’atc gct gag ctt gaa gtc gaa 3’

pyrH reverse 5’gga tgt ttg cac tct gga 3’

pyrP forward 5’gtc gga atg agt cct gct 3’

pyrP reverse 5’ caa taa gga aat cag ccc 3’

pyrR EMSA forward 5’ctt taa tgg cca acc gct tca 3’

pyrR EMSA reverse 5’cag cgc ccg tct aat tgc ctg 3’

pyrR forward 5’agg att gct cac gaa atg 3’

pyrR reverse 5’ tac cgg aat atc tgc acc 3’

rapI EMSA forward 5-gca gtc tgg att gtt tgg gta-3’

rapI reverse 5’-cgt gac taa gtc gta cgg aat-3’

rapI BamHI reverse 5’ cgg gga tcc ttc agc tat tcg ata agc 3’

rapI HindIII forward 5’ gcc aag ctt ttg cgg ggt gtt ttc tta 3’

rnc EMSA forward 5’taa cga aaa tca tcg tag atc 3’

rnc EMSA reverse 5’ttt atc ttt ata atg tga gtg 3’

rnc forward 5’-caa gaa cgg att tcg gtt cac-3’

rnc reverse 5’- cct ttc att atc ttc ata cgg-3’

rplJ forward 5’-cgc gga ctt aac gtt tct gaa-3’

rplJ reverse 5’-aag ctc agc ttg ttc aac cgc-3’

sigW forward 5’- gcg gac atc gta gat att-3’

sigW reverse 5’-aga ata cat ggt caa gcc-3’

sipW forward 5’- tca gtt ctg tca ggt tcg atg-3’

sipW reverse 5’-aac aat tct gtg ggt gac cgc-3’

spoIIE EMSA forward 5’tac ggt tca tac ccg tga ggt 3’

spoIIE EMSA reverse 5’tgg ccc gtt cac tct tct ttc 3’

tasA forward 5’-aag ccg gga gat aag ttg aca -3’

tasA reverse 5’-ctg gct gag gaa atc ttc tgg-3’

tig EMSA forward 5’tta cga gat gca cgg gat tga 3’

tig EMSA reverse 5’cgt taa aac gcc ttc gtt gcc 3’

tig forward 5’- caa gtt tca att cct gga ttc-3’

tig reverse 5’- agg gta ttc tac agg aag agg-3’

trkA(czcO) EMSA forward 5’tgg tct atc aca agc gga ttg 3’

trkA(czcO) EMSA reverse 5’ttg acc agc ccc gat tac tat 3’

trkA(czcO) forward 5’ ata gta atc ggg gct ggt 3’

trkA(czcO) reverse 5’ tcc ttc aag atg cat tcc 3’

trpE EMSA forward 5’gac aga tgt ccc tca gga tca 3’

trpE EMSA reverse 5’gct gtc ctc taa aaa tgc gga 3’

trpE forward 5’ gag aag ctt gac agg gag 3’

trpE reverse 5’ agg aat gcc aag ctc agg 3’

tsf EMSA.forward 5’cat ccc tat cat cgg tat cgt 3’

tsf EMSA reverse 5’cat gcc cgc gcc agt ttt ttc 3’

tsf forward 5’ gcg tta act gaa act gac gga 3’

tsf reverse 5’ tgc aag aag gtg gtc agc taa 3’

ybfO EMSA forward 5’cga gaa gtg tgc tgg ccg atc 3’

ybfO EMSA reverse 5’cag cgg cag ggt cat tct cac 3’

ybfO forward 5’ gaa cga cat gca cag cct 3’

ybfO reverse 5’ atc agg aaa gcc gga ttc 3’

yceC EMSA forward 5’tgc tta aat gaa tca aaa ggc 3’

yceC EMSA reverse 5’atc aat tcg ttg acc ttt ttc 3’

yceC forward 5’ aaa ttg atg gtc ggt ctc 3’

yceC reverse 5’ cag gtt gtc gcc tgt atg 3’

yceF forward 5’ ggt ttg atc ggt tcc ctt 3’

yceF reverse 5’cac ctt gat cca cca gaa 3’

ydjM EMSA forward 5’aaa ggg tct cgc aca ctc ttt 3’

ydjM EMSA reverse 5’tcc tac taa gat aaa agc ggc 3’

yhdN EMSA forward 5’gct gtg atc ata gga gtg aat 3’

yhdN EMSA reverse 5’ggc ttc tat tcc tgt atc tgc 3’

yhdO forward 5’ atc gcg tgt aca cat tcc 3’

yhdO reverse 5’ cgg cgt ttt aat act gct 3’

yhfE reverse 5'-tcc cga ggc ggt ttc aat ttg-3’

yhfE forward 5’-gaa acg gtt cgg aac cac aag -3’

yhfE EMSA forward 5’ ggg tca tca gaa tat ttc tga 3’

yhfE EMSA reverse 5’aat gag ctc cat cgt ttt acg 3’

yhfF forward 5’- ctg att cag cag atc ctt gcc-3’

yhfF reverse 5’-aat tcg gtc tcc att gcc ctc-3’

ylpC EMSA forward 5’aaa caa agc gga atg ccg gaa 3’

ylpC EMSA reverse 5’ttc ctg gcg ttc tct ctt att 3’

ylpC forward 5’ gat gaa gaa cta gcg ggt 3’

ylpC reverse 5’ aat gga tat cgc ctg atc 3’

ylxM EMSA forward 5’ tgt ccg taa ttg att tta ccg 3’

ylxM EMSA reverse 5’ cag ata att cat tct cgt tgt 3’

yneN EMSA forward 5’tgc gga tac act cgg ctt aac 3’

yneN EMSA reverse 5’cat gat aag cag gat ccc tgc 3’

yneN forward 5’ gtc ggt tat acg gga tgg 3’

yneN reverse 5’ ttg cag ctt ttc cat cgc 3’

yokD forward 5’aat ggt gga gct gtt gc 3’

yokD reverse 5’ ggc tgg cat act ttc tct 3’

yokD EMSA reverse 5’tgg aaa agt tgt act ttc aac-3’

yokD EMSA forward 5’gta gct ata cta aga gag caa-3’

yqxM forward 5’ gcc gca ata tgc tta caa-3’

yqxM reverse 5’ctt aag ttt ctc acc tgt-3’

yqxM EMSA forward 5’gtg cca aag acg aga aga gat-3’

yqxM EMSA reverse 5’ctt cgc ctt ttg ctg att gtg-3’

ytsC forward 5’- ggc gaa ttc gtc agt att-3’

ytsC reverse 5’-gat cga taa agg cag aag-3’

ytsC EMSA forward 5’tcc aaa aga tgt acc gcg tat-3’

ytsC EMSA reverse 5’ctg ttt att cag ctt gtt tcc-3’

ytsD forward 5’ gtc acg ctg cag tat gat-3’

ytsD reverse 5’ tac ccc gat cgc taa tga 3’

yuaB forward 5’- gca cct aca gct tct ttc-3’

yuaB reverse 5’-aaa tcc gct tgg caa tgt-3’

yuaB forward EMSA 5’-gat cag ctg gaa agc tct-3’

yuaB reverse EMSA 5’-ccc gag act taa tgc act-3’

yuaF EMSA forward 5’ aat ggt caa gaa ctt ccc gta 3’

yuaF EMSA reverse 5’ cat tgt ttg tat agg tac tcc 3’

yuaF forward 5’ ccg aca tta gtg ctc tca 3’

yuaF reverse 5’tcc tct gag atc atc ttc 3’

yukE EMSA forward 5’ttc taa gaa agt cat cgg agg 3’

yukE EMSA reverse 5’tcc tgc cat att cct cat tac 3’

yukE forward 5’atg gca cag gag gta atg 3’

yukE reverse 5 aag gtt tga gct gct cgt 3’

yvaW (sdpA) BamHI forward 5’ gcc gga tcc ttg atg cca aca ttg ccg aga 3'

yvaW (sdpA) EcoRI reverse 5’ cgg gaa ttc aat gtt ttc ttc tgt agg gct 3’

yvaW (sdpA) forward 5’ agc aat att tca cct cag aa 3’

yvaX (sdpB) forward 5’-aga agt tta ctt ggt ttc tca-3’

yvaX (sdpB) reverse 5’-agc ggt agg gat ata gac att-3’

yvcA reverse 5’ tgt ctt agg ttc att cgc-3’

yvcA forward 5’ gaa gag gaa cca gga tat-3’

yvcA EMSA forward 5’gct tgt cct gct cag aga-3’

yvcA EMSA reverse 5’ act gca tcc tcc tgt cag-3’

yvcE(cwlO) reverse 5’ gtt gct tgt atc aag cgc 3’

yvcE(cwlO) forward 5’ gca tcg gcg gaa aca tta 3’

yvcE EMSA forward 5’-cac gct taa gaa aaa tga caa-3’

yvcE EMSA reverse 3’-agc caa acc aag tgt aat taa-3’

yveN EMSA forward 5’aaa cct cat tca ttt gtc cgg 3’

yveN EMSA reverse 5’atc aac agt cgc gca aaa caa 3’

yveN forward 5’- aag ctg ccg tat gtg gat gag-3’

yveN reverse 5’- gtg cgc tgt gtc cag cac ctt-3’

yveO Forward 5’-tgc gat gat gcg tca aca-3’

yveO Reverse 5’-aac cac ctg ata gtg tcg gtg-3’

yvfA EMSA forward 5’att cta caa tca cta cgg ctt 3’

yvfA EMSA reverse 5’cgt gag att cgc gct gaa att 3’

yvfA forward 5’ ctc acg gct ttt ctc ttg tct 3’

yvfA reverse 5’ ata cgc att tgc ttt ctc ccg 3’

yvfE forward 5’gaa gaa cag ctg gca gaa cga 3’

yvfE reverse 5’ cat att cca cgt atc agg ctc 3’

yvfF forward 5’ cgc tgg aat cca gac aat ttc 3’

yvfF reverse 5’cgt ccg ctt gag gtt gtc ttt 3’

yvfI BamHI reverse 5’ cgg gga tcc aat atc ccg aaa gca cat 3’

yvfI HindIII forward 5’ gcc aag ctt atg aaa cag gga gaa ggc 3’

yvfqI EMSA forward 5’tat gcc gct aat gct ttc ggc 3’

yvqI EMSA reverse 5’gac gga gat ccc aaa tac aat 3’

yvqI forward 5’ atc ggg agc ttg atg aca 3’

yvqI reverse 5’ aga agc cgg ttc agg atc 3’

ywcD EMSA forward 5’cat gcc gac ata taa agt ctt 3’

ywcD EMSA reverse 5’cac aat ggt tgt gaa aac ccc 3’

ywcD forward 5’ gtt gcc gct tgg att ttg 3’

ywcD reverse 5’ ctg gcc gac aag aat gat 3’

ywfB EMSA reverse 5' gat cgc gga tcc tta tgc gta ctc act gct tgt 3'

ywfB EMSA forward 5' gct atg cag ctg tcg gat 3'

ywfH EMSA forward 5’ ccc ttg act ggc tcc cat aac 3’

ywfH EMSA reverse 5’ agc cac ggt tta atc ggc cgc 3’

ywfH HindIII Forward 5’ gca agc ttt ttt ccc tcg tca tta att 3’

ywfH BamHI Reverse 5’ cat ccc att cat cat act gtt tgt 3’

yybL EMSA forward 5’ata aca gcc ttt ata ctt tta 3’

yybL EMSA reverse 5’aca taa aat taa tag tag tct 3’

yybL forward 5’ ttc aac cag aac cac act 3’

yybL reverse 5’ acg ctt aac cat cct cat 3’

yybN forward 5’ gta cct tat ggc tat ggt 3’

yybN reverse 5’ tcc ctg gct att tgc atg 3’

yybN EMSA reverse 5’ tat gcc taa agc agc cgc aag 3’

yybN EMSA forward 5’ctc aat tga gca aat cgc cca 3’

yydF EMSA forward 5’atg ggg taa gca aga gct taa 3’

yydF EMSA reverse 5’ttc taa gtt ttt cac agt ctc 3’

yydG forward 5’gag tta gta act gaa ttt gca 3’

yydG reverse 5’act tat agt cag tgc tat cac 3’

Information of the *B. subtilis* PY79A variant used in the current study, i.e. presence of the ICEBs1 region.

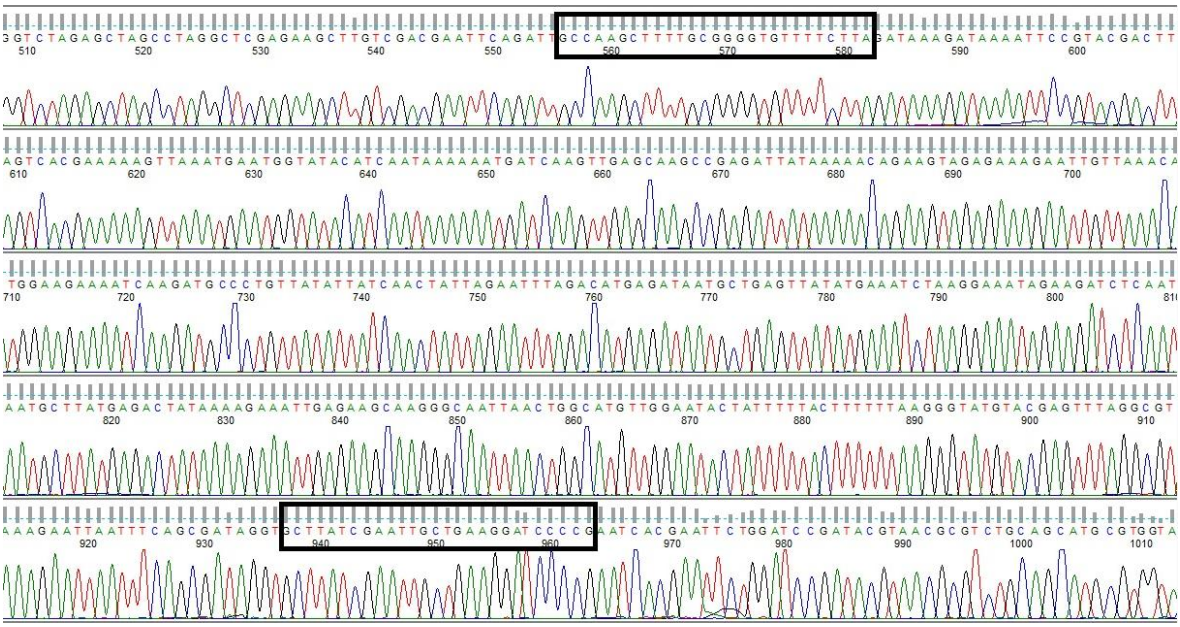
Previously, common *B. subtilis* PY79 strain was reported to lack the ICEBs1 region on its chromosome (Auchtung et al., 2005). However, our microarray results suggested the *rapI* and *phrI* genes described in the ICEBs1 region are highly upregulated in the *lutR* mutant strain (TEK1). Subsequently, as a further confirmation, to construct *rapI-lacZ* transcriptional fusion strain, a 389-bp-long *rapI* gene fragment was amplified from the genome of our lab strain PY79 by PCR with the following specific primers: RapI F (5‘ -GCC AAGCTT TTG CGG GGT GTT TTC TTA- 3’) and RapI R (5’ -CGG GGATCC TTC AGC TAT TCG ATA AGC- 3’). The resulting PCR product was cloned into pGEMT vector for sequencing. As shown in Fig. S4, our sequencing results revealed that our amplified region sequence were identical in sequence with the *rapI* gene present in *B. subtilis* 168 genome. After sequence confirmation, the 389-bp-long *rapI* gene fragment was subcloned in to pMUTIN T3 vector ***rapI-lacZ*** transcriptional fusion was created in our laboratory strain PY79 and used in further experiments. As mentioned above this region is missing in other PY79 strains examined before. This prompted us to examine our PY79 laboratory stock used in the current study. The PY79 laboratory stock that was used in our study has been obtained in 1985 from the laboratory of Arnold L. Demain (Fermentation Microbiology Laboratory, Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts) gifted by Philipe Youngman. To describe our PY79 strain, we have characterized the strain using various diagnostic PCR reactions. To confirm the presence of W23 island in *panB* and *sacA* regions in our stock PY79 strain, we amplified a 396 bp DNA region stretching from 1082 bp to 1478 bp in *panB* gene and the 524 bp DNA fragment between 1402 bp and 1926 bp of the *sacA* gene using PCR reaction and the resulting fragments were cloned into pGEMT vector for sequencing. The sequenced *sacA* and *panB* regions showed similar sequence to PY79 (Fig. S5 and Fig. S6).

Further, the 17 kb DNA region from *ydzA* to *ydaQ* was shown to be absent in the genome of strain PY79 (Zeigler et al., 2008). Therefore, we tested for the presence of *topB* gene locus using PCR reaction (arbitrary selected a gene located within the region between *ydzA* to *ydaQ* in *B. subtilis* 168). The following primer set was used to amplify the *topB* gene: topB F (5’-GTCGACGGCATGACGCTGACG-3’), topB R (5’-CAGACTGCTGAGAACGAGCGG-3’). Our PCR assays indicated that our laboratory stock PY79 strain similar to 1A147 (PY79 BGSC) likely do not contain *ydzA* to *ydaQ* region since the 480 bp fragment of the *tapB* gene could not be amplified from their genomes while this region was successfully amplified from the genome of strain 168 (Fig. S7). Thus, these additional results supported that our stock strain is originated from PY79.

Whether our laboratory stock originates from a stock during the creation of PY79 before ICEBs1 was cured, or the ICEBs1 region originates from another laboratory strain that possesses this region (i.e. ICEBs1 can be transferred between strains by conjugation) is unknown. Based on these experiments, we described our laboratory stock strain as PY79(ICEBs1+), thus as PY79 variant that contains the ICEBs1 region

Auchtung, J. M., Lee, C. A., Monson, R. E., Lehman, A. P. & Grossman, A. D. (2005). Regulation of a *Bacillus subtilis* mobile genetic element by intercellular signaling and the global DNA damage response. *Proc Natl Acad Sci USA* **102**, 12554-12559.

Fig. S4 The DNA sequence chromatogram and blastn analysis of the 389 bp fragment of *rapI* gene amplified from our PY79(ICEBs1+) strain. DNA sequencing was performed by using an automated model 3730 DNA analyzer and BigDye Terminator kit (Applied Bipsystems, Inc). A 389-bp-long *rapI* gene fragment was amplified from the genome of our lab strain primers RapI F and RapI R (see sequence above), and labeled with a box on the chromatogram.



Target reference sequence used for blastn: *Bacillus subtilis* subsp. subtilis str. 168 complete genome
Sequence ID: emb|AL009126.3|

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Query  565     TTGCGGGGTGTTTTCTTAGATAAAGATAAAATTCCGTACGACTTAGTCACGAAAAAGTTA  624
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Sbjct  547306  TTGCGGGGTGTTTTCTTAGATAAAGATAAAATTCCGTACGACTTAGTCACGAAAAAGTTA  547365

Query  625     AATGAATGGTATACATCAATAAAAAATGATCAAGTTGAGCAAGCCGAGATTATAAAAACA  684
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  547366  AATGAATGGTATACATCAATAAAAAATGATCAAGTTGAGCAAGCCGAGATTATAAAAACA  547425

Query  685     GAAGTAGAGAAAGAATTGTTAAACATGGAAGAAAATCAAGATGCCCTGTTATATTATCAA  744
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Query  745     CTATTAGAATTTAGACATGAGATAATGCTGAGTTATATGAAATCTAAGGAAATAGAAGAT  804
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Query  805     CTCAATAATGCTTATGAGACTATAAAAGAAATTGAGAAGCAAGGGCAATTAACTGGCATG  864
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Query  865     TTGGAATACTATTTTTACTTTTTTAAGGGTATGTACGAGTTTAGGCGTAAAGAATTAATT  924
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Sbjct  547606  TTGGAATACTATTTTTACTTTTTTAAGGGTATGTACGAGTTTAGGCGTAAAGAATTAATT  547665
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Query  925     TCAGCGATAGGTGCTTATCGAATTGCTGAA  954
               ||||||||| |||||||||||| ||||||
Sbjct  547666  TCAGCGATAAGTGCTTATCGAATAGCTGAA  547695
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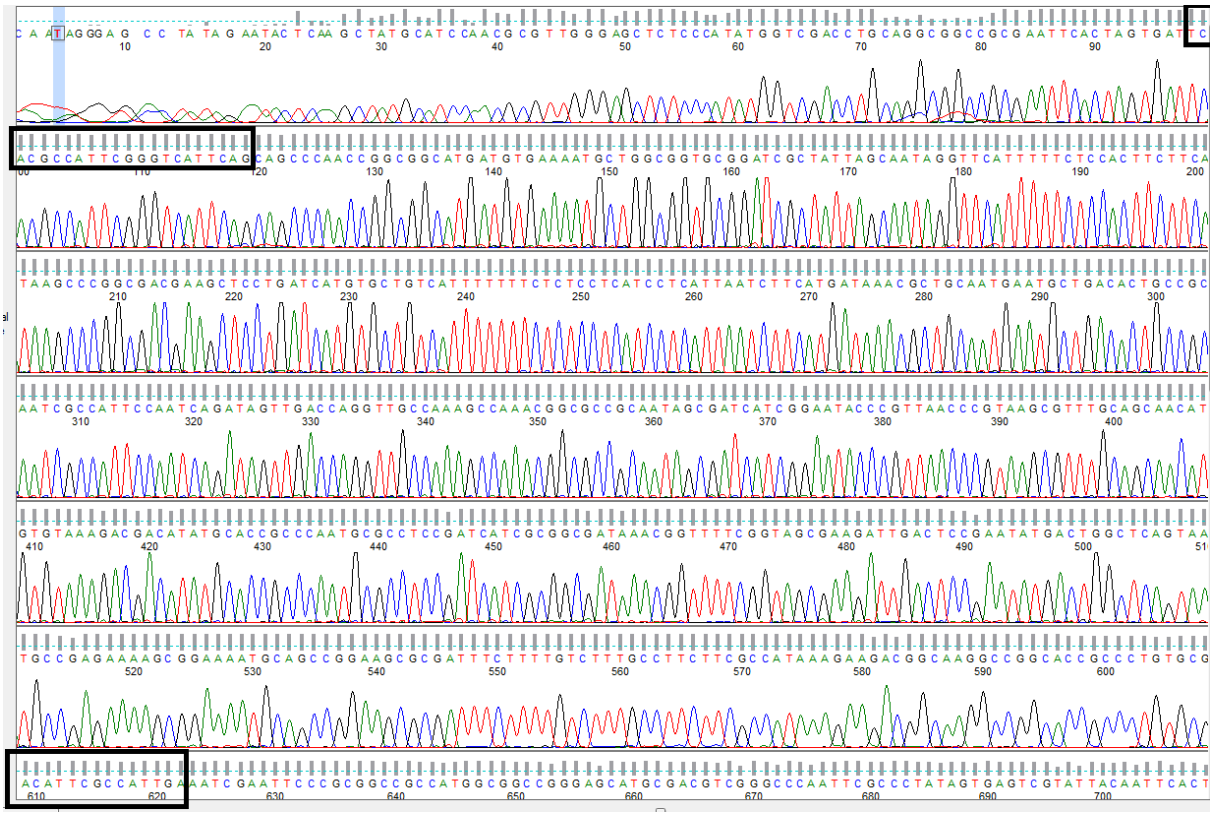
Fig. S5. The DNA sequence chromatogram and blastn analysis of the 524 bp long fragment of the *sacA* gene amplified from our PY79(ICEBs1+) strain. DNA sequencing was performed using an automated model 3730 DNA analyzer and BigDye Terminator (Applied Bipsystems, Inc). The 524 bp long *sacA* gene fragment was amplified from the genome of strain PY79(ICEBs1+) by PCR with the following specific primers: sacA F (5'-TCACGCCATTCGGGTCATTCA-3'), sacA R (5'-TCAATGGCGAATGTCGCACAG-3') as labeled with a box on chromatogram.



Target reference sequence used for blastn: *Bacillus subtilis* strain PY79, sucrase-6-phosphate hydrolase (*sacA*), PTS sucrose-specific enzyme IIBC component (*sacP*), putative formate/nitrite transporter (*ywcJ*), sacPA operon antiterminator (*sacT*), and hypothetical protein YwcI (*ywcI*) genes, complete cds; Sequence ID: gb|EU146093.1|

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Query   64   TCACGCCATTCGGGTCATTCAGCAGCCCAACCGGCGGCATGATGTGAAAATGCTGGCGGT  123
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1402   TCACGCCATTCGGGTCATTCAGCAGCCCAACCGGCGGCATGATGTGAAAATGCTGGCGGT  1461

Query  124   GCGGATCGCTATTAGCAATAGGTTCATTTTTCTCCACTTCTTCATAAGCCCGGCGACGAA  183
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1462   GCGGATCGCTATTAGCAATAGGTTCATTTTTCTCCACTTCTTCATAAGCCCGGCGACGAA  1521

Query  184   GCTCCTGATCATGTGCTGTCATTTTTTTCTCTCCTCATCCTCATTAATCTTCATGATAAA  243
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  244   CGCTGCAATGAATGCTGACACTGCCGCAATCGCCATTCCAATCAGATAGTTGACCAGGTT  303
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Sbjct 1582   CGCTGCAATGAATGCTGACACTGCCGCAATCGCCATTCCAATCAGATAGTTGACCAGGTT  1641
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Query  304  GCCAAAGCCAAACGGCGCCGCAATAGCGATCATCGGAATACCCGTTAACCCGTAAGCGTT  363
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Sbjct 1642  GCCAAAGCCAAACGGCGCCGCAATAGCGATCATCGGAATACCCGTTAACCCGTAAGCGTT  1701

Query  364  TGCAGCAACATGTGTAAAGACGACATATGCACCGCCCAATGCGCCTCCGATCATCGCGGC  423
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1702  TGCAGCAACATGTGTAAAGACGACATATGCACCGCCCAATGCGCCTCCGATCATCGCGGC  1761

Query  424  GATAAACGGTTTTCGGTAGCGAAGATTGACTCCGAATATGACTGGCTCAGTAATGCCGAG  483
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1762  GATAAACGGTTTTCGGTAGCGAAGATTGACTCCGAATATGACTGGCTCAGTAATGCCGAG  1821

Query  484  AAAAGCGGAAAATGCAGCCGGAAGCGCGATTTCTTTTGTCTTTGCCTTCTTCGCCATAAA  543
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1822  AAAAGCGGAAAATGCAGCCGGAAGCGCGATTTCTTTTGTCTTTGCCTTCTTCGCCATAAA  1881

Query  544   GAAGACGGCAAGGCCGGCACCGCCCTGTGCGACATTCGCCATTGA  588
              ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1882  GAAGACGGCAAGGCCGGCACCGCCCTGTGCGACATTCGCCATTGA  1926
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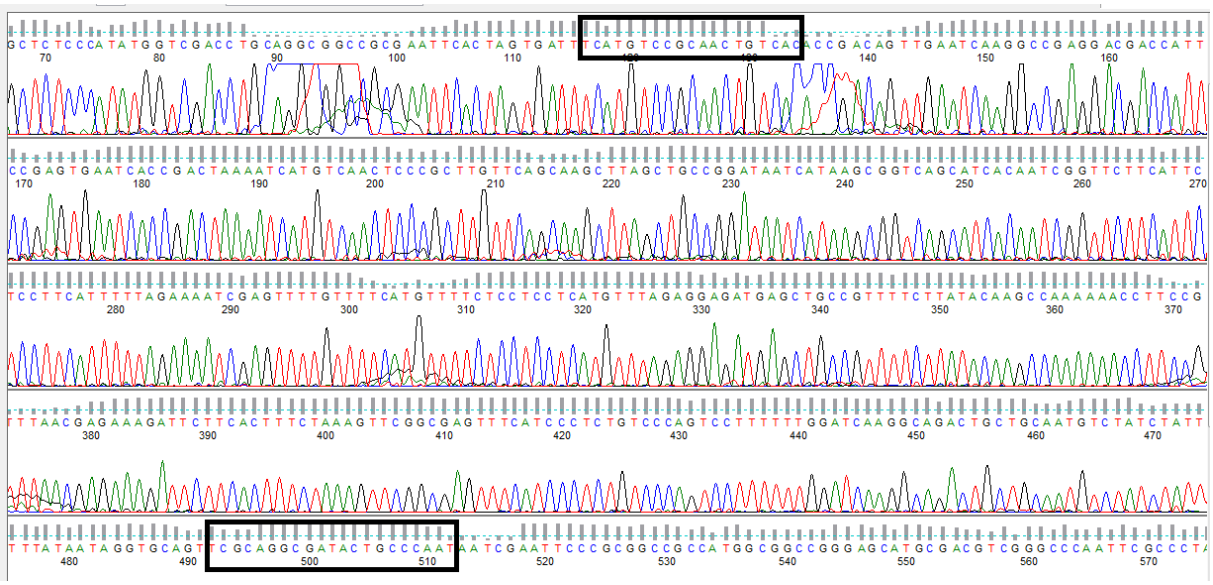
Fig. S6. The DNA sequence chromatogram (above) and blastn analysis (below) of a 396 bp long fragment of the *panB* gene amplified from our PY79(ICEBs1+) strain. DNA sequencing was performed by using an automated model 3730 DNA analyzer and BigDye Terminator (Applied Bipsystems, Inc). The 396 bp long *panB* gene fragment was amplified from the genome of strain PY79(ICEBs1+) by PCR with the following primer sets: panB F (5'-TCATGTCCGCAACTGTCACAC-3'), panB R (5'-ATTGGGCAGTATCGCCTGCGA-3') and labeled with a box on chromatogram.



Target reference sequence used for blastn: *Bacillus subtilis* strain PY79 pantothenate synthetase (*panC*) gene, partial cds; ketopantoate hydroxymethyltransferase (*panB*) gene, complete cds; and transcriptional regulator and biotin acetyl-CoA-carboxylase synthetase (*birA*) gene, partial cds; Sequence ID: gb|EF191505.1

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Query  82    TCATGTCCGCAACTGTCACACCGACAGTTGAATCAAGGCCGAGGACGACCATTCCGAGTG 141
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1082   TCATGTCCGCAACTGTCACACCGACAGTTGAATCAAGGCCGAGGACGACCATTCCGAGTG  1141

Query  142   AATCACCGACTAAAATCATGTCAACTCCCGCTTGTTCAGCAAGCTTAGCTGCCGGATAAT  201
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Sbjct 1382  CATCCCTCTGTCCCAGTCCTTTTTTGGATCAAGGCAGACTGCTGCAATGTCTATCTATTT  1441

Query  442   TTATAATAGGTGCAGTTCGCAGGCGATACTGCCCAAT  478
              ||||||||||||||||||||||||||||||||||||
Sbjct  1442  TTATAATAGGTGCAGTTCGCAGGCGATACTGCCCAAT  1478
```

Fig. S7. PCR analysis of the *topB* gene using chromosomal DNA isolated from our PY79(ICEBs1+) strain (lane 1), 1A147 (PY79 BGSC) (lane 2) and 168 (lane 3) strains of *B. subtilis* as template. Lane 4 is the negative control (no template was added) and lane M denotes the DNA marker (MassRuler reverse DNA ladder Mix obtained from Thermo Scientific).