

Supplemental Information

**Chromosome Translocation Inflates *Bacillus*
Forespores and Impacts Cellular Morphology**

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Table S1. Related to STAR Methods. *B. subtilis* PY79 strains used in this study

Strain	Genotype or description	Reference, source or construction ^a
PY79	Wild type	(Youngman et al., 1984)
BER69	<i>iLE-ssrA*</i> Ωkan <i>amyE::PsspE(2G)-sspB^{E_c}</i> Ωcat	JLG680→JLG963 (Km)
BER991	<i>rpsB-GFP*</i> Ωkan	pEBR226→PY79 (Km)
EBS606	<i>cotC::cat::PspoIIQ-CFPΩtet</i>	(Becker and Pogliano, 2007)
JLG364	<i>xylA::sspB^{E_c}</i> Ωcat	(Lamsa et al., 2016)
JLG451	<i>spoIIIE-GFP-ssrA*</i> Ωkan	(Yen Shin et al., 2015)
JLG453	<i>thrC::PspoIID-sspB^{E_c}</i> Ωspc spoIIIE-sfGFP-ssrA*Ωkan	(Yen Shin et al., 2015)
JLG454	<i>amyE::PspoIIQ-sspB^{E_c}</i> Ωcat thrC::PspoIID-sspB ^{E_cΩspc spoIIIE-sfGFP-ssrA*Ωkan}	(Yen Shin et al., 2015)
JLG680	<i>iLE-ssrA*</i> Ωkan	pJLG113→PY79 (Km)
JLG719	<i>iLE-ssrA*</i> Ωkan <i>xylA::sspB^{E_c}</i> Ωcat	JLG680→JLG364 (Km)
JLG963	<i>amyE::PsspE(2G)-sspB^{E_c}</i> Ωcat	pJLG82→PY79 (Cm)
JLG2059	<i>iLE-ssrA*</i> Ωkan <i>cotC::cat::PspoIIQ-CFPΩtet</i>	EBS606→JLG680 (Tet)
JLG2060	<i>iLE-ssrA*</i> Ωkan <i>amyE::PsspE(2G)-sspB^{E_c}</i> Ωcat cotC::cat::PspoIIQ-CFPΩtet	EBS606→BER69 (Tet)
JLG2144	<i>spoIIIM::Tn917ΩHU287 spoIIIE^{ATP-}</i>	KP519→KP541 (Em)
JLG2618	<i>amyE::PsspE(2G)-sspB^{E_c}</i> Ωcat cotC::cat::PspoIIQ-CFPΩtet	EBS606→JLG963 (Tet)
JLG2648	<i>rpsB-GFP*</i> Ωkan <i>spoIIIE^{ATP-}</i>	BER991→KP541 (Km)
KP107	<i>amyE::PsspE(2G)-lacZΩcat</i>	Kit Pogliano strain collection
KP519	<i>spoIIIM::Tn917ΩHU287</i>	(Sandman et al., 1987)
KP541	<i>spoIIIE^{ATP-}</i> (G467S; ATPase mutant)	(Sharp and Pogliano, 1999)

^a Plasmid or genomic DNA employed (right side the arrow) to transform an existing strain (left side the arrow) to create a new strain are listed. The drug resistance used for selection of transformants is noted in parentheses.

Table S2. Related to STAR Methods. Plasmids constructed for this study

Plasmid	Description
pER226	<i>rpsB-GFPΩkan</i>
pJLG38	<i>GFPΩkan</i>
pJLG82	<i>amyE::PsspE(2G)-sspB^{E_c}Ωcat</i>
pJLG113	<i>ileS-ssrAΩkan</i>

Table S3. Related to STAR Methods. Oligonucleotides used in this study

Primer	Sequence ^a
JLG-7	AATTGGGACAACTCCAGTG
JLG-77	GCTAGCAGCGCAAGCGC
JLG-86	5'P-TAAATGAGAGAGGAAGAAAACGG
JLG-87	5'P-TCATTATACAGTTCATCCATGCC
JLG-95	CATGGATTACGCGTTAACCC
JLG-96	GCACTTTCGGGGAAATGTG
JLG-184	GCTAGCGCAGCAAATGATG
JLG-213	GGATCCATGGATTGTCACAG
JLG-215	AGATCCGAATTCTCATGTTG
JLG-267	<i>ctgtgacaaatccatggatccGCTGAAGTTATTGAGTTAGCC</i>
JLG-268	<i>caaacatgagaattcgatctTAATGGAGAAGAACGGTGG</i>
JLG-422	<i>gggttaacgcgtaatccatgGTCATGAAACAGCTTGGTGC</i>
JLG-423	<i>catcatttgctgcgttagcTTTTGATAGTATTTCAACGATTTC</i>
JLG-424	<i>cactggagttgtcccaattcTAATGCAAAAGAGGCCGG</i>
JLG-425	<i>cacattccccgaaaagtgcTTGTCCCTTGTATAACGCTG</i>
oER304	<i>gggttaacgcgtaatccatgGCTTGAAGCTGGTGTTCACTTC</i>
oER306	<i>cactggagttgtcccaattCCTATTCAAAGGTGATAAGAGGGAC</i>
oER307	<i>cacattccccgaaaagtgcGTTAATACACCGATGCGGCC</i>
oER308	<i>gcgcattgcgtcttagcCGCAGTTGTTCTGTTCTG</i>

^aIn capital letters are shown the regions of the primer that anneals to the template. Homology regions for Gibson assembly are shown in lowercase italics.

Table S4. Related to STAR Methods. Plasmid construction

Plasmid	Description of construction
pER226	This plasmid was constructed by assembling the following 4 fragments by Gibson Assembly (New England Biolabs): (i) the last 712 bp of <i>rpsB</i> coding sequence (not including the stop codon) amplified with primers oER304 and oER308 from genomic DNA of <i>B. subtilis</i> PY79; (ii) <i>GFPΩkan</i> fragment amplified with primers JLG-7 and JLG-77 from pJLG38 (Yen Shin et al., 2015); (iii) a fragment of 598 bp corresponding to the region immediately downstream of the <i>rpsB</i> stop codon, amplified with primers oER306 and oER307 from genomic DNA of <i>B. subtilis</i> PY79; and (iv) a DNA fragment encompassing the spectinomycin resistance gene, the origin of replication, and the ampicillin resistance gene from pDG1662 (Guérout-Fleury et al., 1996), amplified with primers JLG-95 and JLG-96
pJLG38	pJLG36 (Yen Shin et al., 2015) was amplified with primers JLG-86 and JLG-87 and religated. Both primers were phosphorylated at the 5' end, providing substrates for the ligation reaction. After religation, the <i>ssrA*</i> is removed and <i>GFP</i> is linked to a kanamycin resistance gene
pJLG82	A fragment of 467 bp containing the <i>sspE(2G)</i> promoter was amplified from genomic DNA of KP107 using the primers JLG-267 and JLG-268, and inserted in pJLG13 (Yen Shin et al., 2015) amplified with primers JLG-213 and JLG-215 by Gibson assembly. After assembly <i>sspB^{Ec}</i> is under de control of <i>sspE(2G)</i> promoter, and the construct can be inserted at the <i>amyE</i> locus
pJLG113	This plasmid was constructed by assembling the following 4 fragments by Gibson Assembly (New England Biolabs): (i) the last 942 bp of <i>ileS</i> coding sequence (not including the stop codon) amplified with primers JLG-422 and JLG-423 from genomic DNA of <i>B. subtilis</i> PY79; (ii) <i>ssrA*Ωkan</i> fragment amplified with primers JLG-7 and JLG-184 from pJLG3 (Yen Shin et al., 2015); (iii) a fragment of 870 bp corresponding to the region immediately downstream of the last aminoacid-coding codon of <i>ileS</i> , amplified with primers JLG-424 and JLG-425 from genomic DNA of <i>B. subtilis</i> PY79; and (iv) a DNA fragment encompassing the spectinomycin resistance gene, the origin of replication, and the ampicillin resistance gene from pDG1662 (Guérout-Fleury et al., 1996), amplified with primers JLG-95 and JLG-96