Supplemental material

Strain construction. Because *B. subtilis* strain NCIB3610 has low transformation ability, mutant alleles were first introduced into domestic strain 168 and then transferred to strain NCIB3610 by transformation using genomic DNA. Details for strain construction are as follows.

***spx*::*kan* and *yjbH::kan*.** Deletion of *spx* was carried out using an overlap-extension PCR technique. A *kan* cassette was amplified from pDG780 (2) by PCR using primers pUC-R and pUC-F. The primer sequences are shown in Table S1. Upstream and downstream regions of *spx* were amplified by PCR using primer pairs *spx*-F1/*spx*-R1 and *spx*-F2/*spx*-R2, respectively. The 5' sequences of *spx*-R1 and *spx*-F2 were complementary to the sequences of pUC-R and pUC-F, respectively. To connect the three PCR fragments, all three products were mixed and used as a template for a second round of PCR using primers *spx*-F1 and *spx*-R2. The resultant PCR products were used for transformation of strain 168. Deletion of *yjbH* was carried out by the same procedure using different primer sets, *yjbH*-F4/*yjbH*-R4 and *yjbH*-F5/*yjbH*-R5. The *spx*::*kan* and *yjbH*::*kan* mutations were then transferred to NCIB3610.

$P_{spac\text{-}hy}$-*eps* operon and $P_{spac\text{-}hy}$-*bslA*. The 5' part of *epsA* was amplified by PCR with primers *yveK*-F4 and *yveK*-R4. PCR products were digested with *Hin*dIII and *Bam*HI and cloned into the same sites of pMutinT3-hy, which is a derivative of pMutin T3 (3) and contains the *spac*-hy promoter (4). The 5' part of *bslA* was amplified by PCR with primers *yua*B-F8 and *yuaB*-R8. PCR products were digested with *Hin*dIII and *Bam*HI, and cloned into the same sites of pMutinT3-hy. Resultant plasmids were used for transformation of *B. subtilis* 168, and Em^r and Lin^r colonies were selected to obtain $P_{spac\text{-}hy}$-*eps* operon and $P_{spac\text{-}hy}$-*bslA* strains. The *erm* gene of the $P_{spac\text{-}hy}$-*bslA* strain was replaced with the *tet* gene using pEm::Tc (5). $P_{spac\text{-}hy}$-*eps* operon and $P_{spac\text{-}hy}$-*bslA* constructs were then transferred to NCIB3610.

P_{xylA}-*tapA* operon. The *xylA* promoter region and the 5' part of *tapA* were cloned into

26 pCA191, which is a Cm^r derivative of pUC19, using In-Fusion cloning kit (Clontech Takara
27 Bio, Kyoto, Japan). The *xylA* promoter region was amplified from plasmid pX (6) by PCR
28 with primers *xylA-yqxM*-F1 and *xylA-yqxM*-R1. Note that the amplified *xylA* promoter region
29 does not include the *xylR* repressor gene. The 5' region of *tapA* was amplified by PCR with
30 primers *xylA-yqxM*-F2 and *xylA-yqxM*-R2. These primers were designed to provide an end
31 homologous to that of the adjacent DNA fragment for In-Fusion cloning. These *xylA* and
32 *tapA* fragments were inserted between *Hin*dIII and *Eco*RI sites of pCA191, in order. The
33 resultant plasmid was used for transformation of *B. subtilis* 168, and Cm^r colonies were
34 selected to obtain the P_{xylA}-*tapA* operon strain. The *cat* gene of the P_{xylA}-*tapA* operon strain
35 was replaced with the *spc* gene using pCm::Sp (5). The P_{xylA}-*tapA* operon construct was then
36 transferred to NCIB3610.
37
38 **REFERENCES**
39 1. Morimoto T, Loh PC, Hirai T, Asai K, Kobayashi K, Moriya S, Ogasawara N. 2002. Six
40 GTP-binding proteins of the Era/Obg family are essential for cell growth in *Bacillus*
41 *subtilis*. Microbiol 148:3539–3552.
42 2. Guérout-Fleury AM, Shazand K, Frandsen N, Stragier P.1995. Antibiotic-resistance
43 cassettes for *Bacillus subtilis*. Gene 167:335-336.
44 3. Moriya, S., E. Tsujikawa, A. K. Hassan, K. Asai, T. Kodama, and N. Ogasawara. 1998.
45 A *Bacillus subtilis* gene-encoding protein homologous to eukaryotic SMC motor protein
46 is necessary for chromosome partition. Mol. Microbiol 29:179-187.
47 4. Quisel JD, Burkholder WF, Grossman AD. 2001. *In vivo* effects of sporulation kinases
48 on mutant Spo0A proteins in *Bacillus subtilis*. J Bacteriol 183:6573-6578.
49 5. Steinmetz M, Richter R. 1994. Plasmids designed to alter the antibiotic resistance
50 expressed by insertion mutations in *Bacillus subtilis*, through *in vivo* recombination.

51 Gene 142:79-83.

52 6. Kim L, Mogk A, Schumann W. 1996. A xylose-inducible *Bacillus subtilis* integration

53 vector and its application. Gene 181:71-76.

54

55 **Table S1** Primers used in this study

Primer	Sequence (5' to 3')
spx-F1	ATTGCTCGAGGTGGAACCTG
spx-R1	GTTATCCGCTCACAATTCCAGCTTGGTGATGTGTATAG
spx-F2	CGTCGTGACTGGGAAAACAGAGAAGCACAGCGTTTGGC
spx-R2	ATTTAGGATATCAGCTGCTG
yjbH-F4	CGAAATGAGTCATCAAACGC
yjbH-R4	GTTATCCGCTCACAATTCTTGGATGACCGTGGCAATGG
yjbH-F5	CGTCGTGACTGGGAAAACGTTTTGGAAGTCTCTCAGCAC
yjbH-R5	CTCCAGAGCCAATCGAAGCC
pUC-F	GTTTTCCCAGTCACGACG
pUC-R	GAATTGTGAGCGGATAAC
yveK-F4	AAGAAGCTTAAGCCAGCCGCGGGAAGGCTGAA
yveK-R4	GGAGGATCCCCGTCTGATTCATGAACCAG
yuaB-F8	AAGAAGCTTAGGGGGAATTTTGTTATGAAAC
yuaB-R8	GGAGGATCCCAATGAAGCTGTAGACTGTG
xylA-yqxM-F1	GATTACGCCAAGCTTGTTTATCCACCGAACTAAGTTGGTG
xylA-yqxM-R1	TGATATGACAATCGTTCCAATAAACTAACTTTATCTCATC
xylA-yqxM-F2	GTTAGTTTATTGGAACGATTGTCATATCAAGTTACAGTG
xylA-yqxM-R2	ACGGCCAGTGAATTCTTACACGTTTGAAGTGAGAC

56