Supporting Information

Plasmids. The P_{spoIIQ}-*sacY*(1-55) fusion plasmid (pZS9) contains the 247-bp *Eco*RI *Bam*HI-digested *spoIIQ* promoter fragment (extending from –200 to +47 relative to the transcription start site) from pEIA86 (ref. 1 and unpublished results). This fragment was first cloned into *Eco*RI *Bam*HI-digested pRB373 (2); the resulting plasmid was digested with *Bam*HI and *Sph*I and ligated to a *Bgl*II *Sph*I-digested fragment containing *sacY*(1-55), derived from a PCR product amplified from pNDY55 (kindly provided by S. Aymerich). This truncated version of *sacY*, *sacY*(1-55), encoding the constitutively active N-terminal portion of SacY, SacY(1-55), was used throughout the study. The primers were designed so that a *Bgl*II site was created upstream of *sacY*(1-55) to facilitate the cloning; the PCR product extended from 22 bp upstream of the *sacY* translation start codon to 19 bp downstream of the stop codon. The sequences of oligonucleotides used as primers in this study are available on request.

The P_{cotE}-*sacY*(1-55) plasmid (pZS10) was constructed in the same way as pZS9, except that the 400-bp *Eco*RI *Bam*HI-digested fragment containing the p1 promoter of *cotE* from pLZ72 (3, 4) was used, and that the *sacY*(1-55) PCR product was inserted at the *Xba*I site (ends filled in with Klenow fragment of DNA polymerase I) of the pRB373 derivative. The orientation of *sacY*(1-55) was verified by PCR and restriction digestion. The P_{sspA}-*sacY*(1-55) plasmid (pZS106) and the P_{sspE}-*sacY*(1-55) plasmid (pZS107) were constructed in the following way. The promoter regions of *sspA* (–175 to –1 relative to the transcription start point) and *sspE* (–163 to –1; ref. 5) were amplified from strain BR151 chromosomal DNA with creation of an *Eco*RI site at the upstream end of the promoter and were cloned into the *Sma*I site of pBluescript. The *Eco*RI fragment containing the promoter was excised from the pBluescript derivative and cloned into *Eco*RI-digested pRB373 (2); the orientations of the two promoters were checked by PCR and by

restriction digestion. A fragment containing *sacY*(1-55) was inserted into the resulting plasmid by using the same method as for pZS9 construction. The P_{gerE}-*sacY*(1-55) (pZS123), P_{comK}-*sacY*(1-55) (pZS135), P_{srfA}-*sacY*(1-55) (pZS136), and P_{comG}-*sacY*(1-55) (pZS137) plasmids were constructed in the same way as pZS9. The promoter regions amplified from strain BR151 chromosomal DNA were the following (relative to the transcription start point): –119 to –1 for P_{gerE} (6); –324 to –1 for P_{comK} (7); –252 to –1 for P_{srfA} (8); and –166 to –1 for P_{comG} (9). *Eco*R1 and *Bam*H1 sites were created at the upstream and downstream ends of the promoters, respectively.

The P_{trpE}-*sacB'-'lacZ* plasmid (pZS2) was constructed by replacing the *Eco*RI-*Bgl*I fragment of pDH32 (10) with the *Eco*RI-*Bgl*I fragment from pIC38 (11). This fragment contains the *trpE* promoter controlling a *sacB'-'lacZ* translational fusion (the *sacB* leader region plus the first 5 codons of *sacB* fused in frame to the 5' end of *lacZ*). The P_{katX}-*sacB'-'lacZ* fusion plasmid (pZS64) was constructed by replacing the *Eco*RI-*Hin*dIII fragment of pZS2 with an *Eco*RI-*Hin*dIII-digested PCR fragment containing P_{katX}-*sacB'-'lacZ.* This PCR fragment was obtained in two steps. First, the *katX* promoter region (–106 to –1; ref. 12) was amplified from strain BR151 chromosomal DNA by using primers designated 1 and 2, and the *sacB'-lacZ'* portion (extending from the natural *sacB* transcription start site [+1] to +593) was amplified from pIC38 by using primers 3 and 4. Primers 2 and 3 were designed to have 14 overlapping nucleotides. Second, these two PCR products were mixed, and the P_{katX}-*sacB'-'lacZ* fragment was amplified by using primers 1 and 4. The primer 1 sequence was designed to create an *Eco*R1 site at the upstream end of the promoter. Primers 2 and 3 were designed so that the 5' end of the *sacB'-lacZ* transcript would be identical to that of the wild-type *sacB*-mRNA (13). The fusion plasmids with P_{cotE}-*sacB'-'lacZ* (pZS57), P_{sspA}-*sacB'-'lacZ* (pZS94), P_{sspE}-*sacB'-'lacZ* (pZS95), P_{gerE}-*sacB'-'lacZ* (pZS120), P_{comK}-*sacB'-'lacZ* (pZS127), P_{srfA}-*sacB'-'lacZ*

(pZS128), and P_{comG}-*sacB'-'lacZ* (pZS129) were made by the same strategy as that used for pZS64; the 5' end of the *sacB'-'lacZ* transcript was designed to be identical to that of the wild-type *sacB* mRNA. The P_{cotE} promoter extended from –79 to –1 of the P1 transcription start site of *cotE* (3, 14). The promoter fragments for $P_{sspA,}$ $P_{sspE,}$ P_{gerE}, P_{comK}, P_{srfA}, and P_{comG} were the same as used above for *sacY* constructions.

The P_{spoIIQ}-*sacB'-'lacZ* plasmid (pZS41) was constructed in the same manner as pZS64, except that the primers 2 and 3 were designed so that the transcription start sites were identical to the +10 site of the artificial *sacB* transcript in pIC38 (11). The P_{spoIIQ} promoter extends from –88 to +9 relative to the *spoIIQ* transcription start site (1). In our hands, expression of *sacB'-'lacZ* was generally undetectable or very poor if the 5' end of the *sacB'-'lacZ* transcript was not identical to that of wild-type *sacB* (as it is for all the constructs described in the previous paragraph) or to that of the artificial *sacB'-'lacZ* transcript that is present in pIC38. This could be caused by interference with the formation of the correct RAT structure as already noted by Aymerich and Steinmetz (11). The single exception to this is P_{spoIIQ}-*sacB'-'lacZ* in pZS41.

Strains. *Bacillus subtilis* strains used are listed in Table 1. Antibiotics, when required, were used at the following concentrations: chloramphenicol, 5 μg/ml; phleomycin, 2 μg/ml; spectinomycin, 75 μg/ml; neomycin, 10 μg/ml; ampicillin, 75 μg/ml.

Immunofluorescence Microscopy. Immunofluorescence staining of bacteria was performed essentially as described (15, 16), using the rabbit polyclonal anti-β-galactosidase antibody (5 Prime 3 Prime) and goat anti-rabbit fluorescein isothiocyanate-conjugated antibody (Jackson Laboratories). Propidium iodide was used as counter stain. The results of immunofluorescence analysis of β-galactosidase localization are shown in Table 2.

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