Protein-protein interaction domains of Bacillus subtilis DivIVA
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**Fig. S1:** Bacterial two hybrid analysis of the DivIVA interaction to MinJ and RacA. (A) The T25 plasmids p25-N-divIVA (full length DivIVA), pSBLH004 (DivIVAΔ11), pSBLH008 (DivIVAΔ20), pSBLH039 (DivIVAΔ21), pSBLH040 (DivIVAΔ26), and pSBLH041 (DivIVAΔ34), were co-transformed with the T18 plasmids pUT18C-divIVA, pUT18C-racA, and pUT18C-minJ in the *E. coli* strain BTH101 and aliquots of the transformation mixture were spotted onto nutrient agar plates containing ampicillin, kanamycin, IPTG and X-Gal (for details see materials and methods section). Images were taken after 40 h of incubation at 30°C. The T25 plasmids pKT25-racA and p25-N-minJ were used as self-interaction controls. (B) Reciprocal bacterial two hybrid experiment (taken from the same plate) in which the T25 plasmids p25-N-divIVA, pKT25-racA, and p25-N-minJ were co-transformed into BTH101 along with pUT18 plasmids containing the DivIVA truncation series. This experiment confirmed the reduced interaction of DivIVAΔ26 and DivIVAΔ34 with full-length DivIVA. However, T25 fusions of RacA and MinJ did not reveal any interactions with DivIVA in this orientation.
Fig. S2: Western blot after blue native PAGE to analyze oligomerization of chimeric DivIVA proteins. Strains expressing chimeric DivIVA proteins (BSN274: +Lm-144-Bs divIVA; BSN278: +Lm-130-Bs divIVA; BSN288: +Lm-104-Bs divIVA; BSN287: +Lm-83-Bs divIVA; BSN316: +Lm-71-Bs divIVA; BSN321: +Bs-57-Lm divIVA) were grown in LB broth supplemented with 0.5% xylose at 37°C and harvested in mid-logarithmic growth phase. Cell extracts were subjected to blue native PAGE, blotted onto a PVDF membrane and DivIVA proteins were immunostained using the anti-DivIVA antiserum. Strains 168 (wt), 4041 (ΔdivIVA) and strains expressing the *B. subtilis* (BSN51) or the *L. monocytogenes* divIVA gene (BSN238) were used as controls. Please note that differences in the apparent molecular weight of the different DivIVA oligomers are explained by pI differences between *Bs* (pI 5.03) and *Lm* DivIVA (pI 4.77). During electrophoresis, which was performed at pH 7.5, *Lm* DivIVA is more negatively charged as compared to *Bs* DivIVA and therefore runs faster through the gel even though its monomer has the higher theoretical molecular weight. Moreover, the pI values of the C-terminal domains of both proteins are nearly identical (*Bs* CTD: 4.93; *Lm* CTD: 4.80) whereas those of the N-terminal lipid binding domains are rather different (*Bs* LBD: 5.27; *Lm* LBD: 4.72). This explains why all chimeras containing the *Lm* LBD reveal the same apparent oligomer molecular weight as *Lm* DivIVA, whereas the *Bs*-57-*Lm* oligomer runs at the same position as *Bs* DivIVA.
**Fig. S3:** A mutation in the tetramerisation domain (R131A) that causes an inactive Bs DivIVA protein. (A) Phase contrast micrographs obtained on cultures that were cultivated in LB broth containing 1 mM IPTG at 37°C during mid-logarithmic growth. Strains used were 168 (wt), 4041 (ΔdivIVA), BSN5 (+divIVA) and BSN313 (+R131A). Scale bar is 5 µm. (B) Western blot on cell extracts of the same set of strains. DivIVA was visualised using the polyclonal rabbit anti-DivIVA antiserum. (C) The divVAR131A mutant (strain BSN313) reveals a sporulation defect. Sporulation was assayed in a plate assay as described in the legend of Fig. 3B.
Fig. S4: Subcellular localisation of chimeric DivIVA proteins in a B. subtilis \( \Delta \text{divIVA} \) background. Strains BSN294 (DivIVA\(^{Lm-71-Bs-GFP}\)), BSN295 (DivIVA\(^{Lm-144-Bs-GFP}\)), BSN296 (DivIVA\(^{Lm-130-Bs-GFP}\)), BSN297 (DivIVA\(^{Lm-83-Bs-GFP}\)), BSN298 (DivIVA\(^{Lm-104-Bs-GFP}\)), and strain BSN372 (DivIVA\(^{Bs-57-Lm-GFP}\)) were grown in LB supplemented with 0.5% xylose. DivIVA localisation patterns were analysed by epifluorescence microscopy (right images), and for orientation, FM5-95 stained images (left panels) were taken in parallel. Scale bar is 5 µm, septal DivIVA-GFP signals are indicated by arrows. All DivIVA-GFP fusions contain the \( \text{gfpA}206\text{K} \) mutation preventing GFP dimerization. (B) Western blot to demonstrate full-length expression of chimeric DivIVA-GFP proteins. Strains expressing chimeric DivIVA-GFP fusions were grown in LB broth containing or not containing 0.5% xylose to mid-logarithmic growth phase, total cellular proteins were isolated and subjected to SDS-PAGE and subsequent Western blotting. DivIVA-GFP fusion proteins were visualised using the polyclonal anti-GFP antiserum. Strains were as in panel A, but BSN373 (expressing \( Lm \) DivIVA-GFP) was also included. For convenience the names of the respective DivIVA chimeras are indicated above the blot. Please note that DivIVA\(^{Bs-57-Lm-GFP}\) shows some proteolytic degradation.
Fig. S5: Mutations of R102 in Bs divIVA cause a div+ spo- phenotype. (A) Phase contrast micrographs obtained on cultures that were cultivated in LB broth containing 1 mM IPTG (where necessary) at 37°C during mid-logarithmic growth. Strains used for this experiment were 168 (wt), 4041 (∆divIVA), BSN356 (+divIVA), BSN357 (+R102K), and BSN358 (+R102E). Scale bar is 5 µm. (B) Western blot on cell extracts of the same set of strains. DivIVA was visualised using the polyclonal rabbit anti-DivIVA antiserum. (C) Sporulation activity of the R102 mutants. Sporulation was assayed in a plate assay as described in the legend of Fig. 3. Please note that the sporulation defect of the phospho-ablative R102K mutant was less pronounced than that of the phospho-mimetic R102E allele.