

## **In silico prediction and validation of novel SigB promoter binding motifs in *Bacillus subtilis* using a promoter-reporter approach in a wt and $\Delta sigB$ background**

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Bacilli can rapidly adapt to stress conditions by activating the SigB mediated general stress response via either the stressosome RsbRST, the bipartite RsbQP, or the two-component RsbKY module. The distribution of genes involved in these modules in 19 *Bacillus subtilis* genomes and 96 Bacilli members was checked via genome mining and phylogenetic reconstruction. An ortholog of RsbK, typically associated with stress sensing in *B. cereus* group members, was encountered in all inspected *B. subtilis*, hinting at a possible alternative SigB induction route and/or extension of the SigB regulon.

Using *B. subtilis* 168 as a model, this study aimed to identify novel genes under the control of SigB. A SigB promoter binding motif (PBM) was reconstructed based on all listed SigB regulon genes on Subtiwiki, and a promoter-reporter approach was used to analyze SigB-dependent expression of eight newly predicted promoters (*PytoQ*, *Ppucl*, *PygaO*, *PywzA*, *PykaA*, *PylaL*, *PyaaI*, and *PgtaB*) in a wt and  $\Delta sigB$  background. Upon exposure to heat, ethanol, and salt, SigB-dependent LacZ activity was observed in at least one of the tested stress conditions for six predicted PBM, including *PytoQ*, that showcases a link of SigB to vitamin B6 metabolism. The LacZ production of *PywzA* and *PygaO* was not affected in all tested conditions despite retaining a conserved SigB PBM.

Future studies are needed to determine whether such putative SigB-dependent genes respond to so far undiscovered stressors or may be activated as part of other signaling systems.