Supplemental material for

Rok regulates yuaB expression during architecturally complex colony development of Bacillus subtilis 168
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Supplemental materials and methods

RNA isolation, preparation of labeled cDNA, hybridization and analysis

DNA-microarrays containing specific oligonucleotides for all 4107 open reading frames of B. subtilis were prepared as described (4). RNA extraction was performed with the Macaloid / Roche method with 2 additional steps of phenol-chloroform washing. Purified RNA concentration was measured using a Nanodrop spectrophotometer at 260 nm. cDNA was synthesized with the Superscript III Reverse Transcriptase kit (Invitrogen) using 20 µg of total RNA as template and 400 U of SuperScript TM III RT. The reaction contained 0.5 mM dATP, dCTP, dGTP, 0.3 mM dTTP and 0.2 mM of aminoallyl-modified dUTP. The reaction was incubated for 16 h at 42 °C. The amino allyl-modified cDNA was purified with the NucleoSpin Extract II kit using 80% ethanol as wash buffer and 0.1 M sodium carbonate solution (Sigma-Aldrich), pH 9.0, as elution buffer. The purified cDNA was labeled with Cy3- or Cy5-monoreactive dye (Amersham Biosciences) and incubated at room temperature in the dark for 90 min. Labeled cDNA was purified with the NucleoSpin Extract II kit again as described by manufacturer. Dye incorporation and cDNA concentration were assessed with a Nano Drop ND-1000 spectrophotometer (NanoDrop Technologies). The labeled cDNA was hybridized to oligonucleotide microarrays in Ambion Slidehyb #1 buffer (Ambion Europe Ltd) at 45 °C for 18–20 h. After hybridization, slides were washed for 5 min at 37 °C in 2× SSC with 0.5% SDS and 2× 5 min at 37 °C in 1× SSC with 0.25% SDS, dipped five times in 1× SSC 0.1% SDS and then dried by centrifugation (2 min, 2,000 rpm). Fluorescent signals were quantified with the ArrayPro analyser, and processed with Micro-Prep (6). Statistical
analysis was performed using CyberT (1). Genes with a Bayes P-value below $10^{-4}$ with at least threefold differential expression were considered to be significantly affected and presented in Table 2. Microarray data has been deposited in Gene Expression Omnibus database (GSE22370).

**Construction of plasmids and strains**

*Insertional mutagenesis of yobB and yoqM genes.* An erythromycin marker of pE194 (2) was inserted into the SacII site of yobB and the SspI site of the yoqM homologous regions that were obtained by PCR using oligos oATK230 (5’-GCATGCCAAGGATAAAG-3’) and oATK231 (5’-CTTCCAGCCAACAATAG-3’) for yobB and oligos oATK211 (5’-TCATCAACGTGGTTGCTAAG-3’) and oATK212 (5’-CTACCGTTTACAAAGAGATG-3’) for yoqM. The antibiotic resistance marker-containing homologous fragments were transformed into *B. subtilis* 168 and double recombinants were selected.

*Overexpression constructs.* The yuaB gene was amplified by PCR using oligos oATK215 (5’-GCAGTCGACAGGGGGAATTTTGTTATG-3’) and oATK216 (5’-GACGGCTAGCTTTACTCTGTTTCTAGTG-3’) digested with SalI and NheI and inserted into the corresponding sites of pDR110 and pDR111 (kind gifts of David Rudner) resulting pDRyuaB1 and pDRyuaB2, respectively. Plasmids were introduced into *B. subtilis* 168, *rok* and yuaB strains using natural competence (7) and double recombinants were selected based on insertion into the amyE locus.

*Promoter-reporter fusion plasmid.* The promoter region of yuaB was amplified by PCR using oATK213 (5’-CGACTCGAGGTATATGAGGGATGAGATG-3’) and oATK214 (5’-GACGAATTCTAATTTGCTTACCGTTTCAACA-3’) and digested with Xhol and EcoRI and inserted into the corresponding sites of pSG1151, containing GFPmut1 (3) resulting in pSGyuaB.
Gel mobility shift assay

DNA fragments of comK and yuaB promoter regions were obtained with oligos pCK2-KFT1 (5) and oATK213 - oATK214, respectively. EMSA experiments were performed as previously described (5). Various amounts of purified B. subtilis Rok protein and 100ng probe were premixed on ice in binding buffer (80 mM Tris HCl pH8, 4 mM EDTA, 20 mM MgCl2, 2 MM DTT, 400 mM KCl, 40 % glycerol) containing 0.5 µg/µl poly(dl-dC) as non-specific DNA and 2 µg/µl BSA. Samples were incubated at 30 °C, and were loaded on 6% polyacrylamide gel after 20 min incubation. Gels were run in 0.5x TBE buffer (0.045 mM Tris, 0.045 mM Boric Acid, 0.011 mM EDTA) at 90 V for 60 minutes and DNA bands were visualized after staining with ethidium bromide.

Reference List


Figure S1

Overnight grown strains were inoculated into the wells of a microtiter plate containing 2xSGG medium (2xSG medium supplemented with 1% glycerol) and incubated at 37°C for 24 hours. Pellicle structure and fluorescence was detected using an Olympus MVX10 macro zoom fluorescence microscope equipped with a PreciseExcite LED fluorescence illumination (470nm), GFP filter set (excitation at 460/480 nm and emission at 495/540 nm) and an Olympus XM10 monochrome camera.
Figure S2

Gel mobility shift assays were performed as described above in the Supplemental materials and methods. Lanes 1-4 and 5-8 contain the DNA fragments for the *yuaB* and *comK* promoter regions, respectively. Lanes 1 and 5 contain the free DNA probe, while lanes 2, 6; 3, 7 and 4, 8 contain 7.25, 36.25 and 72.5 nM of purified Rok protein, respectively. Increasing amount of Rok protein up to 2 μM final concentration caused no mobility shift of the DNA probe for the *yuaB* promoter (not shown). Empty triangle symbolizes free probes, while solid triangles mark DNA-protein complexes.