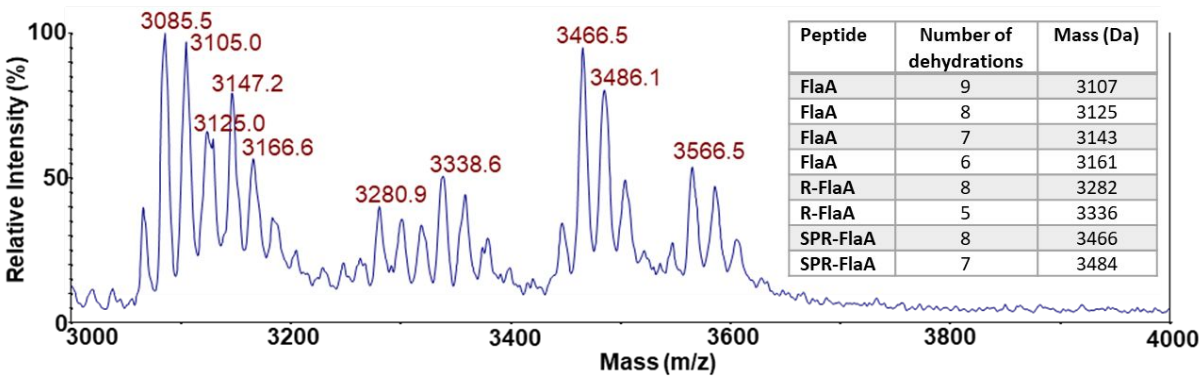
# Mini*Bacillus* PG10 as a convenient and effective production host for lantibiotics

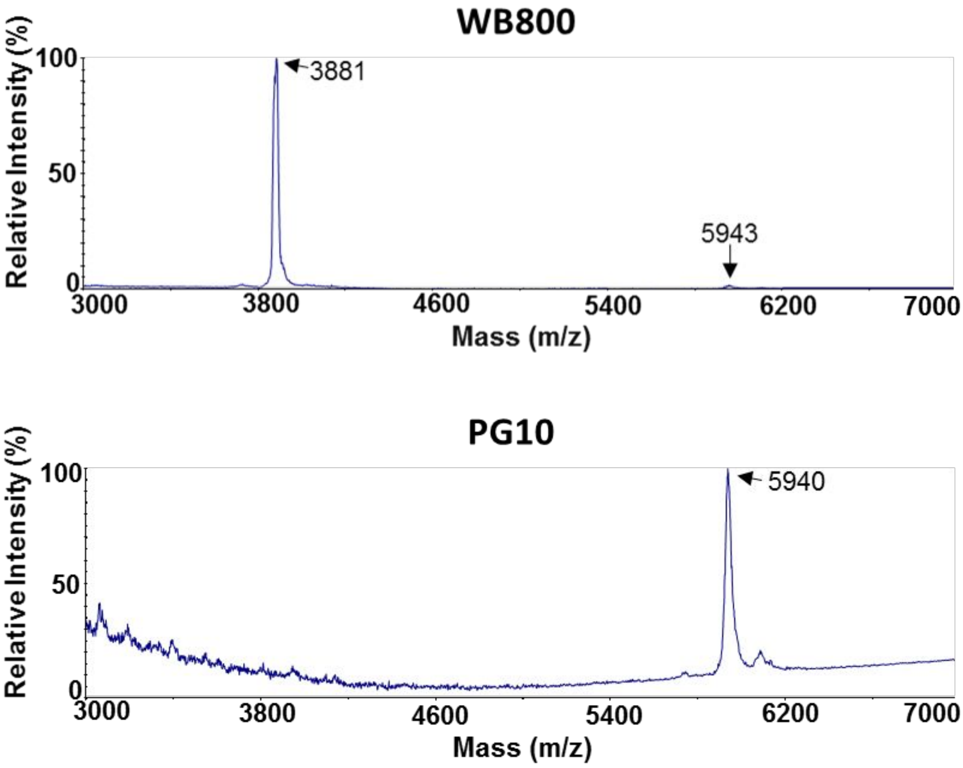
**Amanda Y. van Tilburg, Auke J. van Heel, Jörg Stülke, Niels A.W. de Kok, Anne-Stéphanie Rueff, Oscar P. Kuipers**

## Supplementary Figures and Tables




| Peptide | Number of dehydrations | Mass (Da) |
|---|---|---|
| FlaA | 9 | 3107 |
| FlaA | 8 | 3125 |
| FlaA | 7 | 3143 |
| FlaA | 6 | 3161 |
| R-FlaA | 8 | 3282 |
| R-FlaA | 5 | 3336 |
| SPR-FlaA | 8 | 3466 |
| SPR-FlaA | 7 | 3484 |

**Supplementary Figure 1. MALDI-TOF MS analysis of TCA-precipitated supernatant of ATCC 6633 *spaS::spaASPR$_L$-flaA.*** The spectrum shows flavucin with different dehydration states and with different parts of the ASPR cleavage site of the subtilin leader peptide.




**Supplementary Figure 2. Production of presubtilin by WB800 and PG10.** The MALDI-TOF MS spectra indicate the presence of fully modified presubtilin without the first methionine (theoretical mass: 5943 Da) in the TCA-precipitated supernatant of WB800 and PG10 expressing *spaBTC* and *spaS*. In addition, sublancin (theoretical mass: 3878 Da) is detected in the supernatant of WB800.

**Supplementary Table 1: Diameters of growth inhibition zones after incubation of PG10-derived lantibiotic precursor peptides with various protease-containing samples.**

| Precursor peptide | Incubated with | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **168** | **ATCC 6633 *ΔspaS*** | **NisP** | **Trypsin** | **AprE** | **WprA** | **Vpr** | **Bpr** | **Epr** |
| **$spa_L$-spaS** | 14.4 | 11.6 | - | - | 10.2 | 11.1 | - | 10.8 | - |
| **$spaASPR_L$-spaS** | 9.0 | - | 15.6 | 10.8 | 6.8 | - | - | 10.8 | - |
| **$spa_L$-nisA** | 8.0 | - | - | - | - | - | - | - | - |
| **$spaASPR_L$-nisA** | - | - | 13.2 | 12.0 | - | - | - | - | - |
| **$spa_L$-flaA** | - | - | - | - | - | - | - | - | - |
| **$spaASPR_L$-flaA** | - | - | - | 8.7 | - | - | - | - | - |

Diameters of observed growth inhibition zones are depicted in mm in the table. ‘’-‘’ indicates no growth inhibition zone observed. Results were obtained by performing the agar diffusion test in which TCA-precipitated culture supernatant of PG10 strains producing various lantibiotic precursor peptides was incubated with different protease-containing samples.

**Supplementary Table 2: List of plasmids and bacterial strains used in this study.**

| Plasmids | Relevant characteristics | Reference |
|---|---|---|
| pJOE8999 | Vector for markerless genetic engineering of *B. subtilis* by employing CRISPR-Cas9; $km^R$ | [1] |
| pJOE_ΔspaS | pJOE8999 derivative for deletion of *spaS* in *B. subtilis* ATCC 6633 | This study |
| pJOE_nisA | pJOE8999 derivative for replacement of *spaS* in *B. subtilis* ATCC 6633 by $spaASPR_L$-*nisA* encoding a hybrid peptide composed of the subtilin leader and nisin core peptide with NisP cleavage site (ASPR) | This study |
| pJOE_flaA | pJOE8999 derivative for replacement of *spaS* in *B. subtilis* ATCC 6633 by $spaASPR_L$-*flaA* encoding a hybrid peptide composed of the subtilin leader and flavucin core peptide with NisP cleavage site (ASPR) | This study |
| pJOE_xylR | pJOE8999 derivative for insertion of *xylR* under control of its native promoter in the *sacA* locus of *B. subtilis* strain PG10 | This study |
| pDG1664 | Integration vector for genomic integration in the *thrC* locus of *B. subtilis* 168; $ery^R$, $spc^R$, $amp^R$ | [2] |
| pDG1664-$P_{xylA}$-spaBTC | pDG1664 derivative containing the xylose inducible promoter ($P_{xylA}$) and *spaBTC* derived from chromosomal DNA of *B. subtilis* ATCC 6633 | This study |
| pDG1664-spaRK | pDG1664 derivative carrying *spaRK* with the native promoter derived from chromosomal DNA of *B. subtilis* ATCC 6633 | Lab collection |
| pDR111 | Integration vector for genomic integration in the *amyE* locus of *B. subtilis* 168 containing the IPTG inducible hyper-spank promoter ($P_{spank-hy}$); $spc^R$, $amp^R$ | [3] |
| pDR111-$P_{spank-hy}$-spaS | pDR111 derivative carrying the structural gene *spaS* | This study |
| pDR111-$P_{spank-hy}$-$spaASPR_L$-spaS | pDR111 derivative carrying the structural gene *spaS* with NisP cleavage site in the subtilin leader peptide (ASPR) | This study |
| pDR111-$P_{spank-hy}$-$spa_L$-nisA | pDR111 derivative carrying the native subtilin leader fused to the nisin core gene *nisA* | This study |
| pDR111-$P_{spank-hy}$-$spaASPR_L$-nisA | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the nisin core gene *nisA* | This study |
| pDR111-$P_{spank-hy}$-$spa_L$-flaA | pDR111 derivative carrying the native subtilin leader fused to the flavucin core gene *flaA* | This study |
| pDR111-$P_{spank-hy}$-$spaASPR_L$-flaA | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the flavucin core gene *flaA* | This study |
| pDR111-$P_{spank-hy}$-spaS-$P_{spank}$-spaBTC | pDR111 derivative carrying the structural gene *spaS* regulated by $P_{spank-hy,}$ and *spaBTC* regulated by $P_{spank}$ | This study |
| pDR111-$P_{spank-hy}$-$spaASPR_L$-spaS-$P_{spank}$-spaBTC | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the subtilin core gene *spaS* regulated by $P_{spank-hy,}$ and *spaBTC* regulated by $P_{spank}$ | This study |
| pDR111-$P_{spank-hy}$-$spa_L$-nisA-$P_{spank}$-spaBTC | pDR111 derivative carrying the native subtilin leader fused to the nisin core gene *nisA* regulated by $P_{spank-hy,}$ and *spaBTC* regulated by $P_{spank}$ | This study |
| pDR111-$P_{spank-hy}$-$spaASPR_L$-nisA-$P_{spank}$-spaBTC | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the nisin core gene *nisA* regulated by $P_{spank-hy,}$ and *spaBTC* regulated by $P_{spank}$ | This study |
| pDR111-$P_{spank-hy}$-$spa_L$-flaA-$P_{spank}$-spaBTC | pDR111 derivative carrying the native subtilin leader fused to the flavucin core gene *flaA* regulated by $P_{spank-hy,}$ and *spaBTC* regulated by $P_{spank}$ | This study |
| pDR111-$P_{spank-hy}$-$spaASPR_L$-flaA-$P_{spank}$-spaBTC | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the flavucin core gene *flaA* regulated by $P_{spank-hy,}$ and *spaBTC* regulated by $P_{spank}$ | This study |
| pDR111-$P_{spaS}$-spaBTC-$P_{spaS}$-spaS | pDR111 derivative carrying the *spaBTC* and *spaS* controlled by their native subtilin-regulated promoters | This study |
| pDR111-aprE | pDR111 derivative carrying *aprE* from chromosomal DNA of *B. subtilis* ATCC 6633 | This study |
| pDR111-wprA | pDR111 derivative carrying *wprA* from chromosomal DNA of *B. subtilis* ATCC 6633 | This study |
| pDR111-vpr | pDR111 derivative carrying *vpr* from chromosomal DNA of *B. subtilis* ATCC 6633 | This study |
| pDR111-epr | pDR111 derivative carrying *epr* from chromosomal DNA of *B. subtilis* ATCC 6633 | This study |
| pDR111-bpr | pDR111 derivative carrying *bpr* from chromosomal DNA of *B. subtilis* ATCC 6633 | This study |

| | | |
|---|---|---|
| pNZE3-nisA | pNZ8048 derivative containing *PnisA-nisA* | Lab collection |
| pNZE3-flaA | pNZ8048 derivative containing *PnisA-flaA* | Lab collection |
| pDG1664-$P_{spank}$-spaBTC | pDG1664 derivative containing the IPTG-inducible promoter $P_{spank}$ and *spaBTC* derived from chromosomal DNA of *B. subtilis* ATCC 6633 | Lab collection |
| | | |
| **Strains** | **Relevant characteristics** | **Reference** |
| ***E. coli*** | | |
| MC1061 | *araD139 Δ(araA-leu)7697 Δ(lac)X74 galK16 galE15(GalS) lambda- e14- mcrA0 relA1 rpsL150(strR) spoT1 mcrB1 hsdR2* | Lab collection |
| DH5α | $F^-$ *endA1 glnV44 thi- recA1 relA1 gyrA96 deoR nupG purB20* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17($r_K^- m_K^+$), $λ^-$ | Lab collection |
| Top10 | *F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara leu) 7697 galU galK rpsL (StrR) endA1 nupG* | Lab collection |
| | | |
| ***B. subtilis*** | | |
| ATCC 6633 | Natural subtilin producer | ATCC collection |
| ATCC comK | ATCC 6633 containing pGSP12 for expression of *comK* | [4] |
| ATCC *ΔspaS* | ATCC 6633 derivative; deletion of *spaS* | This study |
| ATCC nisA | ATCC 6633 derivative;<br>*spaS::spaASPR*$_L$*-nisA* | This study |
| ATCC flaA | ATCC 6633 derivative;<br>*spaS::spaASPR*$_L$*-flaA* | This study |
| 168 | $trp^-$ | Lab collection |
| 168 spaS | 168 derivative;<br>*thrC::*$P_{xylose}$*-spaBTC*<br>*amyE::*$P_{spank-hy}$*-spaS* | This study |
| 168 nisA | 168 derivative;<br>*thrC::*$P_{xylose}$*-spaBTC*<br>*amyE::*$P_{spank-hy}$*-spaASPR*$_L$*-nisA* | This study |
| 168 flaA | 168 derivative;<br>*thrC::*$P_{xylose}$*-spaBTC*<br>*amyE::*$P_{spank-hy}$*-spaASPR*$_L$*-flaA* | This study |
| WB800 | Eight-fold protease-deficient strain; *ΔnprE ΔnprB ΔaprE Δepr Δmpr Δbpr Δvpr ΔwprA* | [5] |
| WB spaS | WB800 derivative;<br>*thrC::*$P_{xylose}$*-spaBTC*<br>*amyE::*$P_{spank-hy}$*-spaS* | This study |
| PG10 | 168 derivative; large-scale genome-minimized strain | [6] |
| PG10 spaS_$P_{xylose}$-spaBTC (P1) | PG10 derivative;<br>*thrC::* $P_{xyl}$*-spaBTC*<br>*amyE::*$P_{spank-hy}$*-spaS*<br>*sacA::xylR* | This study |
| PG10 spaS_$P_{spank}$-spaBTC (P2) | PG10 derivative;<br>*amyE::*$P_{spank-hy}$*-spaS-*$P_{spank}$*-spaBTC* | This study |
| PG10 spaS_SURE (P3) | PG10 derivative;<br>*thrC::spaRK*<br>*amyE::*$P_{spaS}$*-spaBTC-*$P_{spaS}$*-spaS* | This study |
| PG10 spaASPR$_L$-spaS | PG10 derivative;<br>*amyE::*$P_{spank-hy}$*-spaASPR*$_L$*-spaS-*$P_{spank}$*-spaBTC* | This study |
| PG10 spa$_L$-nisA | PG10 derivative;<br>*amyE::*$P_{spank-hy}$*-spa*$_L$*-nisA-*$P_{spank}$*-spaBTC* | This study |
| PG10 spaASPR$_L$-nisA | PG10 derivative;<br>*amyE::*$P_{spank-hy}$*-spaASPR*$_L$*-nisA-*$P_{spank}$*-spaBTC* | This study |
| PG10 spa$_L$-flaA | PG10 derivative;<br>*amyE::*$P_{spank-hy}$*-spa*$_L$*-flaA-*$P_{spank}$*-spaBTC* | This study |
| PG10 spaASPR$_L$-flaA | PG10 derivative;<br>*amyE::*$P_{spank-hy}$*-spaASPR*$_L$*-flaA-*$P_{spank}$*-spaBTC* | This study |
| | | |
| PG10 AprE | PG10 derivative; | This study |

| | | |
|---|---|---|
| | *amyE::P*$_{spank\text{-}hy}$*-aprE* | |
| PG10 WprA | PG10 derivative;<br>*amyE::P*$_{spank\text{-}hy}$*-wprA* | This study |
| PG10 Vpr | PG10 derivative;<br>*amyE::P*$_{spank\text{-}hy}$*-vpr* | This study |
| PG10 Epr | PG10 derivative;<br>*amyE::P*$_{spank\text{-}hy}$*-epr* | This study |
| PG10 Bpr | PG10 derivative;<br>*amyE::P*$_{spank\text{-}hy}$*-bpr* | This study |
| | | |
| ***Micrococcus luteus*** | Indicator strain | Lab collection |

**Supplementary Table 3: List of oligonucleotides used in this study.**

| Primer name | Nucleotide sequence (5'-3') |
|---|---|
| sgRNA-encoding sequence for *spaS* | agtgaatcactttgtacacc |
| sgRNA-encoding sequence for *sacA* | ggacgattttggatacatgt |
| ΔspaS_up-fw | ttggcctagacggccactggtcacctcctttcaatacct |
| ΔspaS_up-rv | atggccttattggccgatctccgccaaacgcagtaaa |
| ΔspaS_down-fw | atggccaacgaggcctttaagcatttgggcggccatt |
| ΔspaS_down-rv | gtggccgtctaggccaagcatcaccttgctctgactcct |
| 77-wprA-F-SalI | gtacgtcgactgcaggagggatatcatgaaacg |
| 79-wprA-R-PaeI | gtcagcatgccgctttttgattacttctcaacaac |
| 81-aprE-F-SalI | ctgcgtcgactaaaaggagagggtaaagaatgag |
| 83-aprE-R-PaeI | gatcgcatgcctgcttctttttatcttattgtg |
| 86-vpr-F-NheI | ctgagctagccacaaagggggaaacacattgaataag |
| 87-vpr-R-PaeI | gatcgcatgccaggaggcatttgcgaattaatctg |
| 105-epr-F-SalI | gcacgtcgacaaggagataaatcatgaaaaac |
| 106-epr-R-NheI | gtcagctagcgttcatggcatgatagcaatag |
| 108-bpr-F-SalI-RBS | gcacgtcgacaaggagggtaagaaatgaggaaaaaaacgaaaaac |
| 109-bpr-R-NheI | gtcagctagcagggatatctgcttaatttgtc |
| 145-pDR111-QF-F | gctagccgcatgcaagctaattc |
| 146-pDR111-QF-R | attcctgcagccctggcgaatg |
| 147-spaBTCS-F | cgccagggctgcaggaatggcctggattgaaaggttcttg |
| 148-spaBTCS-R | agcttgcatgcggctagcaattgtgttcctccttaaagcc |
| 206-spaI-F-SfiI | gtaggccaaggaggccaccaatatgtcaaagttcgatg |
| 207-nisA-R-SfiI | tgaggcctatctggcctcatttgaaacatgaattgaac |
| 208-flaA-R-SfiI | tgaggcctatctggccttattttccgattgtgaatgaac |
| 209-spaS_up-F-SfiI | tgaggccaacgaggcctggcatattccatctcaacatc |
| 210-spaS_up-R-SfiI | ggtggcctccttggcctacctaaatagtaacagacaaatatc |
| 211-spaS_down-F-SfiI | tgaggcctgattggccttagcatcaccttgctctgac |
| 212-spaS_down-R-SfiI | tgaggccttattggccatgataaagttgatcgtcatag |
| 275-sacA1-Fw-Eco | gcagggtctctgattgtcaccgggtttgttgc |
| 276-sacA1-Rv-Eco | gcagggtctctagctgcccttccaataaatcac |
| 277-xylr-Fw-Eco | gcagggtctctagctaagtacatcacctattagtttg |
| 278-xylr-Rv-Eco | gcagggtctctcgactcatatgagaaggtgccatgtc |
| 279-sacA2-Fw-Eco | gcagggtctctgtcggccgcctcgattataac |
| 280-sacA2-Rv-Eco | gcagggtctctgtagtatcttgcactcaagacctcttc |
| 281-pJOE_sacA-Eco | gcagggtctctctacgccgtcgaccctatagtgagtcg |
| 282-pJOE_sacA-Eco | gcagggtctctaatcgccaataaggcctttctagattaag |
| 291_fw_Pspank | acgtggtctcaacaagtattcaaatatatcctcctcac |
| 333-pDR-Eco_fw | gtcaggtctcatatattcacacccgtttgctag |
| 334-pDR-Eco_rv | gtcaggtctcattgtaactctttcacacccgtcgtt |
| 375-pDR-Phs-bb-fw-Eco | gtcaggtctcatataaggaagataaatcccataag |
| 376-pDR-Phs-bb-rv-Eco | gtcaggtctcattgttctcgctttgattgttctatc |
| 335-pDG-Ps-BTC-Eco-rv | tcgaggtctcatatatcggatcctagaagcttatcg |
| ASRo224 | tctcttgccagtcacgttac |
| ASRo241 | gctcggatccctcgtttccaccgaattagc |
| ASRo254 | cgtcgctagcaaggagattgactaatatgaaatccttatatacacctacag |
| ASRo255 | ccgagcggccgctttttttagggaagggaggctctcacctcccttcccttttttattaaattaataatgcttttgtccaatc |
| ASRo256 | gctcaagcttattgaaaggaggtgaccaatatg |
| ASRo257 | gctcgctagcaaacgggtgtgaatataaac |
| ASRo288 | ggagtacaaagtgaaattgaagtaatacgtgggctagcttttgagtcttgtttagagactttcacaa |
| ASRo289 | ggagtacaaagtgaaattgaagtaatttgcggagtgatttttgagtcttgtttag |
| ASRo290 | attacttcaatttcactttgtactccaggttgtaaaact |
| ASRo291 | gctcgcatgctctgctcccgcccttatgggatttatcttc |
| ASRo292 | ggagtgcataatgatttagatgtaatacgtgggctagcttttgagtcttgtttagagactttcacaacatcc |
| ASRo293 | ggagtgcataatgatttagatgtaatttgcggagtgatttttgagtcttgtttag |
| ASRo294 | attacatctaaatcattatgcactccaggttgtat |
| ASRo297 | gtgtacaaagtgattcacttttccaacgtgggctagcttttgagtcttgtttagagactttcacaac |
| ASRo298 | tggaaaagtgaatcactttgtacaccaggatgtgtaac |

## References


(1) Altenbuchner, J. (2016) Editing of the *Bacillus subtilis* Genome by the CRISPR-Cas9 System. *Appl. Environ. Microbiol. 82*, 5421–5427.

(2) Guérout-Fleury, A. M., Frandsen, N., and Stragier, P. (1996) Plasmids for ectopic integration in *Bacillus subtilis*. *Gene 180*, 57–61.

(3) Gonzalez-Pastor, J. E., Britton, R. A., Losick, R., Fawcett, P., Grossman, A. D., Monson, R., and Eichenberger, P. (2002) Genome-Wide Analysis of the Stationary-Phase Sigma Factor (Sigma-H) Regulon of *Bacillus subtilis*. *J. Bacteriol. 184*, 4881–4890.

(4) Duitman, E. H., Wyczawski, D., Boven, L. G., Venema, G., Kuipers, O. P., and Hamoen, L. W. (2007) Novel methods for genetic transformation of natural *Bacillus subtilis* isolates used to study the regulation of the mycosubtilin and surfactin synthetases. *Appl. Environ. Microbiol. 73*, 3490–3496.

(5) Wu, S. C., Yeung, J. C., Duan, Y., Ye, R., Szarka, S. J., Habibi, H. R., and Wong, S. L. (2002) Functional production and characterization of a fibrin-specific single-chain antibody fragment from *Bacillus subtilis*: Effects of molecular chaperones and a wall-bound protease on antibody fragment production. *Appl. Environ. Microbiol. 68*, 3261–3269.

(6) Reuß, D. R., Altenbuchner, J., Mäder, U., Rath, H., Ischebeck, T., Sappa, P. K., Thürmer, A., Guérin, C., Nicolas, P., Steil, L., Zhu, B., Feussner, I., Klumpp, S., Daniel, R., Commichau, F. M., Völker, U., and Stülke, J. (2017) Large-scale reduction of the *Bacillus subtilis* genome: Consequences for the transcriptional network, resource allocation, and metabolism. *Genome Res. 27*, 289–299.