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

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Supplementary Figures and Tables
Supplementary Figure 1. MALDI-TOF MS analysis of TCA-precipitated supernatant of ATCC 6633 spaS::spaASPR₆-flaA. The spectrum shows flavucin with different dehydration states and with different parts of the ASPR cleavage site of the subtilin leader peptide.

| 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                       | 3456      |
| SPR-FlaA | 7                       | 3484      |
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 |
| spaL-spaS         | 14.4 | 11.6 | - | - | 10.2 | 11.1 | - | 10.8 | - |
| spaASPRL-spaS     | 9.0 | - | 15.6 | 10.8 | 6.8 | - | - | 10.8 | - |
| spaL-nisA         | 8.0 | - | - | - | - | - | - | - | - |
| spaASPRL-nisA     | - | - | 13.2 | 12.0 | - | - | - | - | - |
| spaL-flaA         | - | - | - | - | - | - | - | - | - |
| spaASPRL-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.

| Plasmid Code         | Relevant characteristics                                                                 | Reference |
|----------------------|-----------------------------------------------------------------------------------------|-----------|
| pJOE8999             | Vector for markerless genetic engineering of *B. subtilis* by employing CRISPR-Cas9, km<sup>+</sup> | 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-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-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<sup>R</sup>, spc<sup>R</sup>, amp<sup>R</sup> | 2         |
| pDG1664-P<sub>flaA</sub>-spaBTC | pDG1664 derivative containing the xylene inducible promoter (P<sub>flaA</sub>) 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<sub>spank-hy</sub>); spc<sup>R</sup>, amp<sup>R</sup> | 3         |
| pDR111-P<sub>spank-hy</sub>-spaS  | pDR111 derivative carrying the structural gene spaS                                      | This study|
| pDR111-P<sub>spank-hy</sub>-spaASPR-spaS  | pDR111 derivative carrying the structural gene spaS with NisP cleavage site in the subtilin leader peptide (ASPR) | This study|
| pDR111-P<sub>spank-hy</sub>-spa-nisA  | pDR111 derivative carrying the native subtilin leader fused to the nisin core gene nisA | This study|
| pDR111-P<sub>spank-hy</sub>-spaASPR-nisA  | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the nisin core gene nisA | This study|
| pDR111-P<sub>spank-hy</sub>-spa-flaA  | pDR111 derivative carrying the native subtilin leader fused to the flavucin core gene flaA | This study|
| pDR111-P<sub>spank-hy</sub>-spaASPR-flaA  | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the flavucin core gene flaA | This study|
| pDR111-P<sub>spank-hy</sub>-spaS-P<sub>spank-hy</sub>-spaBTC  | pDR111 derivative carrying the structural gene spaS regulated by P<sub>spank-hy</sub> and spaBTC regulated by P<sub>spank</sub> | This study|
| pDR111-P<sub>spank-hy</sub>-spaASPR-spaS-P<sub>spank-hy</sub>-spaBTC  | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the subtilin core gene spaS regulated by P<sub>spank-hy</sub> and spaBTC regulated by P<sub>spank</sub> | This study|
| pDR111-P<sub>spank-hy</sub>-spa-nisA-P<sub>spank-hy</sub>-spaBTC  | pDR111 derivative carrying the native subtilin leader fused to the nisin core gene nisA regulated by P<sub>spank-hy</sub> and spaBTC regulated by P<sub>spank</sub> | This study|
| pDR111-P<sub>spank-hy</sub>-spaASPR-nisA-P<sub>spank-hy</sub>-spaBTC  | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the nisin core gene nisA regulated by P<sub>spank-hy</sub> and spaBTC regulated by P<sub>spank</sub> | This study|
| pDR111-P<sub>spank-hy</sub>-spa-flaA-P<sub>spank-hy</sub>-spaBTC  | pDR111 derivative carrying the native subtilin leader fused to the flavucin core gene flaA regulated by P<sub>spank-hy</sub> and spaBTC regulated by P<sub>spank</sub> | This study|
| pDR111-P<sub>spank-hy</sub>-spaASPR-flaA-P<sub>spank-hy</sub>-spaBTC  | pDR111 derivative carrying the ASPR-modified subtilin leader fused to the flavucin core gene flaA regulated by P<sub>spank-hy</sub> and spaBTC regulated by P<sub>spank</sub> | This study|
| pDR111-P<sub>spaS</sub>-spaBTC-P<sub>spaS</sub>-spaBTC  | 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|
| pNZ8048 derivative containing PnisA-nisA | Lab collection |
| pNZ8048 derivative containing PnisA-flaA | Lab collection |
| pDG1664-P<sup>spank</sup>-spaBTC | pDG1664 derivative containing the IPTG-inducible promoter P<sup>spank</sup> 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 galK16 galE15(GalS) lambda- e14- mcrA0 relA1 rpsL150(strR) spoT1 mcrB1 hsdR2 | Lab collection |
| DH5α | F<sup>−</sup> endA1 glnV44 thi- recA1 relA1 gyrA96 deoR purB20 φ80lacZΔM15 Δ(lacZYA-argF′)U169, hsdR17(rK<sup>−</sup> mK<sup>+</sup>) | Lab collection |
| Top10 | F<sup>−</sup> 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; spaS::spaASPR<sup>L</sup>-nisA | This study |
| ATCC flaA | ATCC 6633 derivative; spaS::spaASPR<sup>L</sup>-flaA | This study |
| 168 | | Lab collection |
| 168 spaS | 168 derivative; thrC::P<sub>xylose</sub>-spaBTC amyE::P<sub>spank-hy</sub>-spaS | This study |
| 168 nisA | 168 derivative; thrC::P<sub>xylose</sub>-spaBTC amyE::P<sub>spank-hy</sub>-spaASPR<sup>L</sup>-nisA | This study |
| 168 flaA | 168 derivative; thrC::P<sub>xylose</sub>-spaBTC amyE::P<sub>spank-hy</sub>-spaASPR<sup>L</sup>-flaA | This study |
| WB800 | Eight-fold protease-deficient strain; ΔnprE ΔnprB ΔaprE Δmpr Δbpr Δvpr ΔwprA | 5 |
| WB spaS | WB800 derivative; thrC::P<sub>xylose</sub>-spaBTC amyE::P<sub>spank-hy</sub>-spaS | This study |
| PG10 | 168 derivative; large-scale genome-minimized strain | 6 |
| PG10 spa<sub>P<sub>xylose</sub></sub>-spaBTC (P1) | PG10 derivative; thrC::P<sub>xylose</sub>-spaBTC amyE::P<sub>spank-hy</sub>-spaS sacA::xylR | This study |
| PG10 spa<sub>P<sub>spank</sub></sub>-spaBTC (P2) | PG10 derivative; amyE::P<sub>spank-hy</sub>-spaS-P<sub>spank</sub>-spaBTC | This study |
| PG10 spa<sub>S</sub>_SURE (P3) | PG10 derivative; thrC::spaRK amyE::P<sub>spank-spaBTC-P<sub>spank</sub>-spaS</sub> | This study |
| PG10 spaASPR<sub>L</sub>-spaS | PG10 derivative; amyE::P<sub>spank-hy</sub>-spaASPR<sub>L</sub>-spaS-P<sub>spank</sub>-spaBTC | This study |
| PG10 spa<sub>-nisA</sub> | PG10 derivative; amyE::P<sub>spank-hy</sub>-spa-<sub>nisA-P<sub>spank</sub>-spaBTC</sub> | This study |
| PG10 spaASPR<sub>L</sub>-nisA | PG10 derivative; amyE::P<sub>spank-hy</sub>-spaASPR<sub>L</sub>-nisA-P<sub>spank</sub>-spaBTC | This study |
| PG10 spa<sub>-flaA</sub> | PG10 derivative; amyE::P<sub>spank-hy</sub>-spa<sub>-flaA-P<sub>spank</sub>-spaBTC</sub> | This study |
| PG10 spaASPR<sub>L</sub>-flaA | PG10 derivative; amyE::P<sub>spank-hy</sub>-spaASPR<sub>L</sub>-flaA-P<sub>spank</sub>-spaBTC | This study |
| PG10 AprE | PG10 derivative; | This study |
|                                       |                                    |
|---------------------------------------|------------------------------------|
| **amyE::P<sub>agank-ly</sub>-aprE**  | PG10 derivative; amyE::P<sub>agank-ly</sub>-aprE |
| **PG10 Wpr**                          | This study                         |
| **PG10 Vpr**                          | This study                         |
| **PG10 Epr**                          | This study                         |
| **PG10 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 | agtgaaacttctttgtaacc                                          |
| sgRNA-encoding sequence for sacA  | ggacagttaacgcttttttcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactgtttgcatacctccaaacagtgaataaatgagtctagtcatgactgaataaatgagtctagtctgactg
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