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**Supplemental Table 1. Strains and plasmids used in this study.**

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***B. subtilis***

|        |  |           |
|--------|--|-----------|
| PY79   | laboratory strain used as a host for transformation                                  |           |
| 3610   | undomesticated wild strain capable of forming robust biofilms                        | (1)       |
| CY819  | $\Delta ywbHG$ in 3610, $Mls^R$  | this work |
| CY881  | $\Delta ysbAB$ in 3610, $Spc^R$  | this work |
| CY882  | $\Delta yxaC$ in 3610, $Kan^R$   | this work |
| CY886  | $\Delta ywbHG$ , $\Delta ysbAB$ , $\Delta yxaC$ in 3610, $Mls^R$ , $Kan^R$ , $Spc^R$ | this work |
| CY1250 | $amyE::P_{hyperspank-ywbHG}$ in 3610, $Spc^R$  | this work |
| FY250  | $amyE::P_{yxaKC-lacZ}$ in 3610, $Cm^R$   | this work |
| FY251  | $amyE::P_{ysbAB-lacZ}$ in 3610, $Cm^R$   | this work |
| FY255  | $\Delta ackA::mIs$ and $amyE::ackA$ in 3610, $Mls^R$ , $Cm^R$                        | this work |
| FY256  | $\Delta pta::mIs$ and $amyE::pta$ in 3610, $Mls^R$ , $Cm^R$                          | this work |
| KG2    | $amyE::P_{epsA-lacZ}$ , $\Delta ywcBA$ in 3610, $Kan^R$ , $Cm^R$                     | this work |
| YC110  | $amyE::P_{epsA-lacZ}$ in 3610, $Cm^R$  | (2)       |
| YC121  | $amyE::P_{tapA-lacZ}$ in 3610, $Spc^R$   | (3)       |
| YC259  | $amyE::P_{ywbH-lacZ}$ in 3610, $Cm^R$  | this work |
| YC535  | $\Delta ywcBA$ in 3610, $Kan^R$  | this work |
| YC566  | $amyE::P_{tapA-lacZ}$ , $\Delta ywcBA$ in 3610, $Kan^R$ , $Spc^R$                    | this work |
| YC1218 | $\Delta acsA::mIs$ in 3610, $Mls^R$  | this work |
| YC1220 | $\Delta ackA::mIs$ in 3610, $Mls^R$  | this work |
| YC1230 | $\Delta pta::mIs$ in 3610, $Mls^R$   | this work |

**Other strains**

HG003 a clinical isolate of *Staphylococcus aureus* that forms robust biofilms (4)

**Plasmid**

|        |   |           |
|--------|---|-----------|
| pYC161 | $amyE::P_{ywbHG-lacZ}$ fusion in pDG268, $Cm^R$ | this work |
| pFY50  | $amyE::P_{yxaKC-lacZ}$ fusion in pDG268, $Cm^R$ | this work |
| pFY51  | $amyE::P_{ysbAB-lacZ}$ fusion in pDG268, $Cm^R$ | this work |

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## Supplemental Table 2. Oligonucleotides used in this study.

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|                       |   |
|-----------------------|---|
| ywcBA-P1              | 5'-acaccggatcaatcttttagcgg-3'                         |
| ywcBA-P2              | 5'-caattcgccctatagtgagtcgt atcagctttgtacatggctt-3'    |
| ywcBA-P3              | 5'-ccagcttttgttcccttttagtgag tccatattggaacaggaataa-3' |
| ywcBA-P4              | 5'-caatcgggttctgctttatacgc-3'                         |
| ywbHG-P1              | 5'-gtcccataaatacgaatttcgtatg-3'                       |
| ywbHG-P2              | 5'-caattcgccctatagtgagtcgt catattgatgcctcccttat-3'    |
| ywbHG-P3              | 5'-ccagcttttgttcccttttagtgag tattgaaaagctggcgaatt-3'  |
| ywbHG-P4              | 5'-aaccgatggccactccctgaagaa-3'                        |
| ysbAB-P1              | 5'-gctttataacctaataatcagcgg-3'                        |
| ysbAB-P2              | 5'-caattcgccctatagtgagtcgt agcactcattttcttcacctc-3'   |
| ysbAB-P3              | 5'-ccagcttttgttcccttttagtgag ggaggataagccaaggctgaa-3' |
| ysbAB-P4              | 5'-ctattctacacaataaccggatc-3'                         |
| yxac-P1               | 5'-ggccaaagccatcgccatgctg -3'                         |
| yxac-P2               | 5'-caattcgccctatagtgagtcgt gactttcacatcaagtgctgc-3'   |
| yxac-P3               | 5'-ccagcttttgttcccttttagtgag atttaacaaagaaagactgcc-3' |
| yxac-P4               | 5'-cgaccaagcgattgctgatgagg-3'                         |
| ywbI-P1               | 5'-gttatcaatgcggaataacggatg-3'                        |
| ywbI-P2               | 5'-caattcgccctatagtgagtcgt catgcttcaccctttcta-3'      |
| ywbI-P3               | 5'-ccagcttttgttcccttttagtgag ggatgatgcaaatatggatg-3'  |
| ywbI-P4               | 5'-ccgagcctctcctgtcagcgcac-3'                         |
| P <sub>ywbH</sub> -F1 | 5'-gtacgaattcctatagacaaaaggaataacttc-3'               |
| P <sub>ywbH</sub> -R1 | 5'-gtacggatcctatatagaagaaggctgtcacc-3'                |
| ackA-F1:              | 5'-gtacggatccgcttttgaagaccggacttg-3'                  |
| ackA-R1:              | 5'-gtacggatccgatttattttgctaaacgaa-3'                  |
| pta-F1:               | 5'-gtacggatccgcttcattcgtcatttgct-3'                   |
| pta-R1:               | 5'-gtacggatccttattacagtgcttgccg-3'                    |
| sda-F:                | 5'-ttaattgggttcctagcatgaga-3'                         |
| sda-R:                | 5'-atacggaaataatagtccgag-3'                           |
| sinR-F:               | 5'-ggccagcgtattaaacaataaccg-3'                        |
| sinR-R:               | 5'-cccattcactatctaattgacca-3'                         |
| codY-F:               | 5'-tccatgctgcaagctgcccag-3'                           |
| codY-R:               | 5'-atattcttcaggaattgacgat-3'                          |

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## Supplemental references

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4. **Herbert S, Ziebandt A-K, Ohlsen K, Schäfer T, Hecker M, Albrecht D, Novick R, Götz F.** 2010. Repair of Global Regulators in *Staphylococcus aureus* 8325 and Comparative Analysis with Other Clinical Isolates. Infection and Immunity **78**:2877-2889.