

Supplementary Materials

Improving Surfactin Production in *Bacillus subtilis* 168 by Metabolic Engineering

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A

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TTTTCAAAAACAGAGTACAGCGACCTTTTAGCAAAAGACAAGGACGAGCAGACAGACTATTTTATCATCTAT
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B

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GTCGATCCCGGCTACAAAATGGCTGTATGCGCCGTACACCCTGATTCCCCGAGGATATCACAATGGTCTCGTA
CGAAGAGCTTTTATTAATA

Figure S1. Nucleotide sequences of the *sfp* gene in *Bacillus subtilis*. **(A)** Nucleotide sequence of the inactive pseudogene *sfp* in wild-type *B. subtilis* 168. **(B)** Nucleotide sequence of the active *sfp* gene.

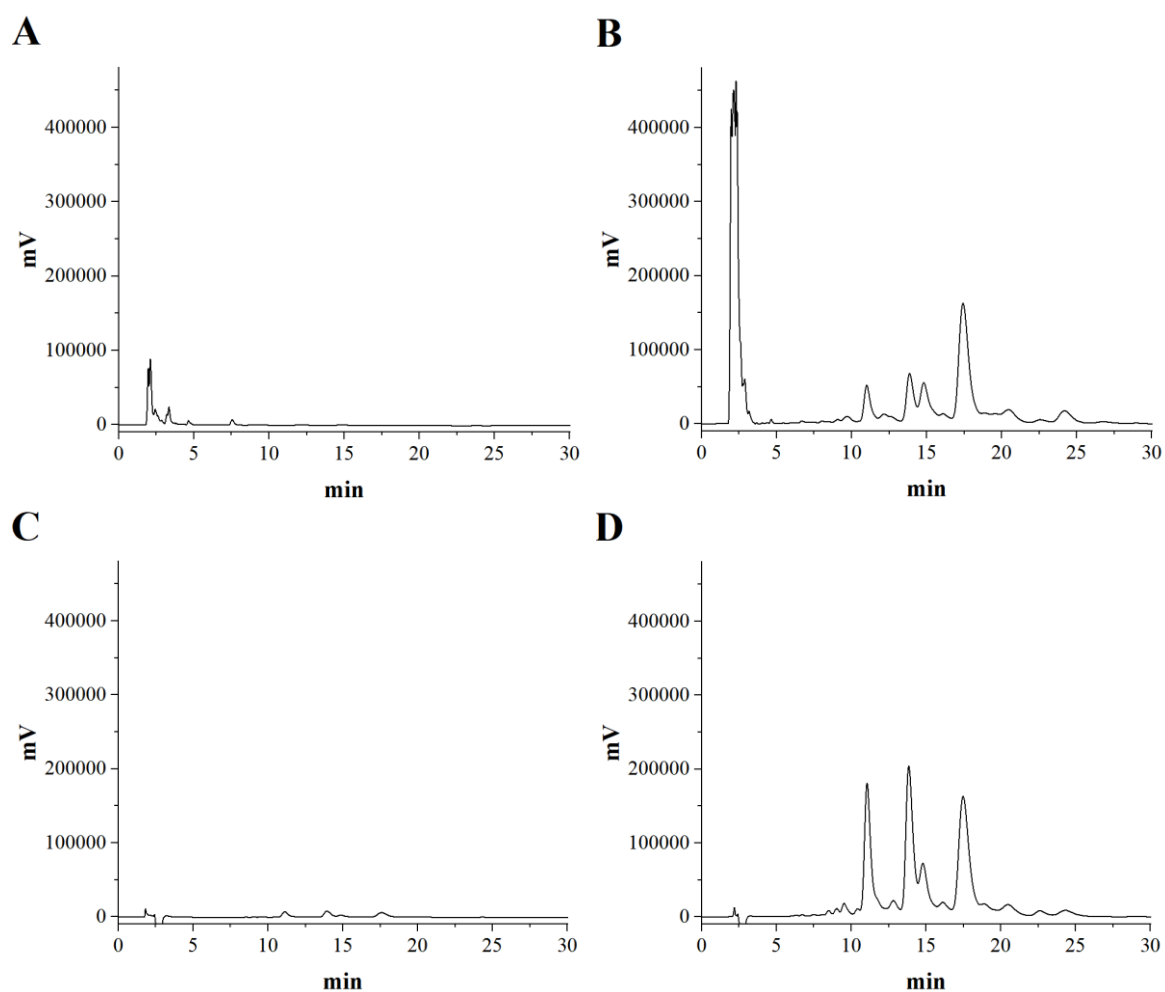


Figure S2. HPLC profiles of the samples and standards. **(A)** HPLC profile of the supernatant of wild-type *B. subtilis* 168 fermented for 48 h, concentrated 4-fold after treatment. **(B)** HPLC profile of the supernatant of recombinant strain BSSF2 fermented for 48 h, concentrated 4-fold after treatment. **(C)** HPLC profile of the 0.2 g/L surfactin standard. **(D)** HPLC profile of the 5.0 g/L surfactin standard.

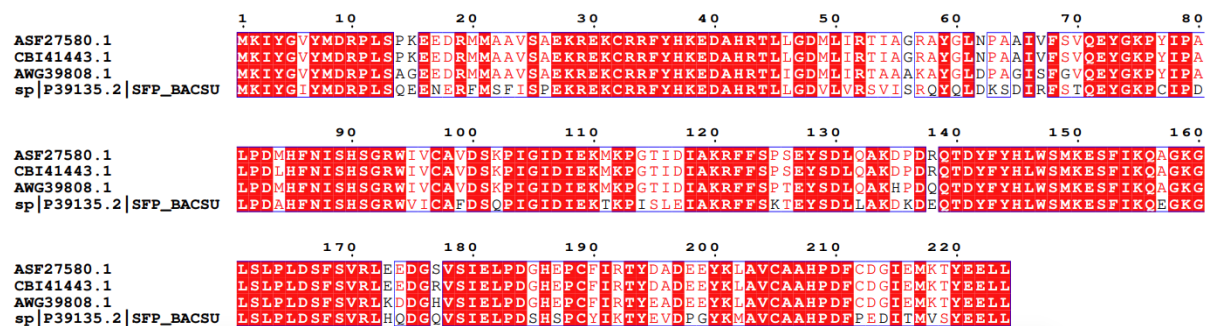


Figure S3. Alignment results of the amino acid sequences of PPTase from different sources. GenBank:

ASF27580.1, PPTase from *Bacillus amyloliquefaciens* MT45; GenBank: CBI41443.1, PPTase from *Bacillus amyloliquefaciens* DSM7; GenBank: AWG39808.1, PPTase from *Bacillus velezensis* BS-37 DSM7; GenBank: P39135.2, active PPTase from *Bacillus subtilis* 168.

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      1      10      20      30      40      50      60      70      80
AIG20548.1  MKTIDSKGLLGNRVYLQVFSAYSILMLGVFIDMLAIMTIVGFWEVDPTMTGLFPVAYALPGITFGSWAGVIADRFRRKIP
CAL0278229.1  ...MEKPLFRNQKGLMTLLASQTISSLGDWLHLLAVLTLAFAQLHASPLDMSLLMSFALPVTLGPVSGLLADRFDRKT

      90      100      110      120      130      140      150      160
AIG20548.1  IMMFCNLMVGLITITALLFVODIHWLLVALMIRSLFIVFYPAQQTLTRQIVSPDLTKKAVSINCIIVEGCTKIVGPLIGGM
CAL0278229.1  IMFLSEIIGRALTTVLSGVVSELWQLYVELSVQSCFSSLELPAGKNGKLELAPEAHIQOAVSVSSIIDNSSKIFGPALGGT

      170      180      190      200      210      220      230      240
AIG20548.1  LLSWFOPEFCLIMRAISCLLATLVLIPTIKFKETISKEFVEKOKOSTWTAWVOCWSYVLSNRITLSTMTIFVTIAMAVLQIL
CAL0278229.1  LIAAFSIHSVFYINAGAFFLSAVILFFLPKRDALFLOKANTPQEKTAALTSTIKECLQFLKRMPLLLTGLLTACVVLFFVQLI

      250      260      270      280      290      300      310
AIG20548.1  VDSGFPTLFLKSLFPHDKSKMGYIITSIIIGGILGALLTKLKQFOYGRVVCGGMVLMGTFGGIGLITFS..TVFVLLAYL
CAL0278229.1  GDSGAIIILIRSFSGAPPELAGWCMAVSCAGMLLTAATGRRRITSYLLYFSAGTLILLGLATGGAPFLSGMGIAGITLFFIF

      320      330      340      350      360      370      380      390
AIG20548.1  ISFIACIGSGGLMLVSNQVILQIESDQDQVGRVFCIQSSTNAVLIISPAMSGLVHLFCVTLQLYVYGCVGLVIGVIGVS
CAL0278229.1  AFIIMGAAPGLVHLIPQILVQTTVPVDYSGRVFGAIQSATTLASILGMAAGGVLAEWIGVSLAFLVCCLLIMIGLIITLI

      400      410
AIG20548.1  LQKYLWAKHKHEPIRANNF..
CAL0278229.1  GKRIAESRRYLVTKSNKGAQG

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Figure S4. Alignment results of the amino acid sequences of KrsE from *Bacillus thuringiensis* (GenBank:

AIG20548.1) and YfiS of *B. subtilis* 168 (GenBank: CAL0278229.1).

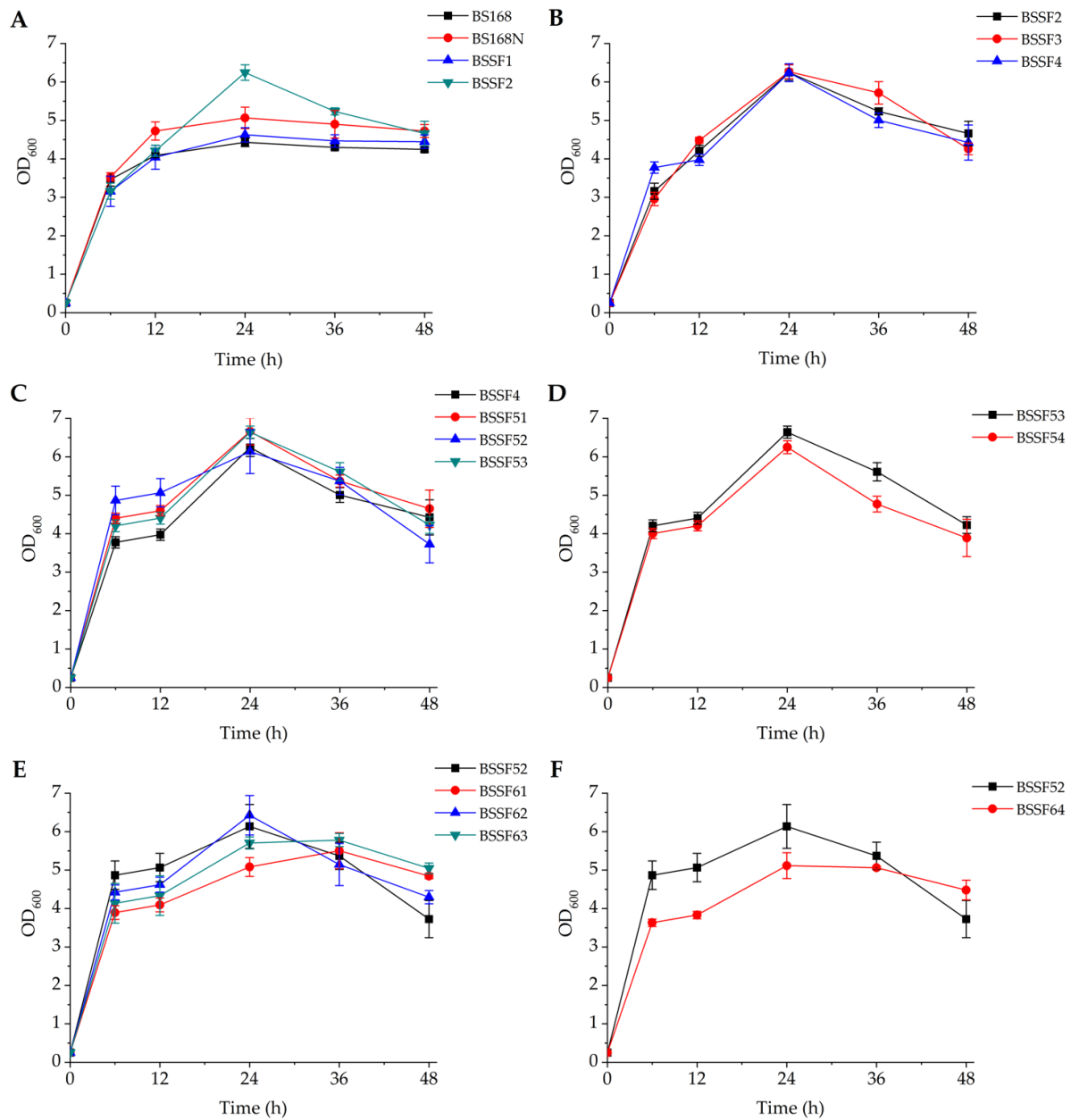


Figure S5. Effects of different gene modifications on bacterial growth. **(A)** Growth curves of wild-type *Bacillus subtilis* 168 (BS168), parental strain BS168N, *yrpC* gene knockout strain BSSF1 and *sfp* gene overexpression strain BSSF2. **(B)** Growth curves of control strain BSSF2, *pssD* gene knockout strain BSSF3 and *yvkC* gene knockout strain BSSF4. **(C)** Growth curves of control strain BSSF4, *yerP* gene overexpression strain BSSF51, *yfiS* gene overexpression strain BSSF52 and *ycxA* gene overexpression strain BSSF53. **(D)** Growth curves of control strain BSSF53 and *ycxA-efp* gene overexpression strain BSSF54. **(E)** Growth curves of control strain BSSF52 and strains BSSF61, BSSF62 and BSSF63 obtained by replacing

the native promoter of the *srfA* operon with promoters P_{HpaII} , P_{43} and P_{SB} , respectively. (F) Growth curves of control strain BSSF52 and *codY* gene knockout strain BSSF64.