

Supplementary File S1

***In vivo* quantification of surfactin nonribosomal
peptide synthetase complexes in *Bacillus subtilis***

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Table S1. Oligonucleotides used in this study

Order	Oligonucleotide name	Sequence (5' → 3')	Application	
1	Vector_ <i>srfAA</i> _F	ACGAGATGCTGGGATCCTCTAGATTTCTATAGTGTCACCT	Amplification of pJOE6743.1 backbone for using as the vector	Construction of pMAV22
2	Vector_ <i>srfAA</i> _R	TCCAGTAAAATCCGTCCCTATAGTGAGTCGTATTAGGCATGC		
3	up_ <i>srfAA</i> _F	CTCACTATAGGGACGGATTTTACTGGAGGATTTCgcc	Amplification of end of <i>srfAA</i> gene with excluding the stop codon	
4	up- <i>srfAA</i> -R	CTTCTCCTTTACTAACCATgaaaatttcattaatttccagctcatccag		
5	eGFP- <i>srfAA</i> -F	ggataaattaatggaattttCATGGTTAGTAAAGGAGAAGAACTTTTCACTG	Amplification of <i>mEGFP</i>	
6	eGFP- <i>srfAA</i> -R	ctcatatgccacctctTTATTTGTATAGTTCATCCATGCCAAGTGTAATC		
7	down- <i>srfAA</i> _F	GATGAACTATACAAATAAagaggtggcatatgagcaaaaaatc	Amplification of downstream region of <i>srfAA</i>	
8	down_ <i>srfAA</i> _R	CTAGAGGATCCCAGCATCTCGTTCAACTTTCACcc		
9	Vector_ <i>srfAB</i> _F	tcTATAGTGAGTCGCCCTATAGTGAGTCGTATTAGGCATGC	Amplification of pJOE6743.1 backbone for using as the vector	Construction of pMAV23
10	Vector_ <i>srfAB</i> _R	TCCAGTAAAATCCGTCCCTATAGTGAGTCGTATTAGGCATGC		
11	up_ <i>srfAB</i> _F	CGACTCACTATAGGGCGACTCACTATAgactttgcttcaggctacatgcag	Amplification of end of <i>srfAB</i> gene with excluding the stop codon	
12	up- <i>srfAB</i> -R	CAGTGAAAAGTTCTTCTCCTTTACTAACCATttttaattctcctcaagcatgtcaagatatctcc		
13	eGFP- <i>srfAB</i> -F	gagatatctttgacatgcttgaggagaatttaaaaATGGTTAGTAAAGGAGAAGAACTTTTCACTG	Amplification of <i>mEGFP</i>	
14	eGFP- <i>srfAB</i> -R	gccttctgtaattcccttgcggtTTATTTGTATAGTTCATCCATGCCAAGTGTAATCC		
15	down- <i>srfAB</i> _F	GATTACACTTGGCATGGATGAACTATACAAATAAaacgcaagggaattacagaagg	Amplification of downstream region of <i>srfAB</i>	
16	down_ <i>srfAB</i> _R	TAGAGGATCCCCGccgtactcaaagtggtagtctgc		

Order	Oligonucleotide name	Sequence (5' → 3')	Application	
17	Vector_ <i>srfAC</i> _F	tgtattgctgtttacgtttgGGGATCCTCTAGATTTCTATAGTGTACC	Amplification of pJOE6743.1 backbone for using as the vector	Construction of pMAV24
18	Vector_ <i>srfAC</i> _R	gctcatccaagaaggtaaagTCCCTATAGTGAGTCGTATTAGGCATGC		
19	up_ <i>srfAC</i> _F	CTATAGGGAActttacctcttgatgagcttcct	Amplification of end of <i>srfAB</i> gene with excluding the stop codon	
20	up- <i>srfAC</i> -R	CTCCTTTACTAACCATtgaaccggttacggttggtattaagaaattc		
21	eGFP- <i>srfAC</i> -F	ccgtaacggtttcaATGGTTAGTAAAGGAGAAGAAGCTTTTCACTG	Amplification of <i>mEGFP</i>	
22	eGFP- <i>srfAC</i> -R	catcacttcatTTATTTGTATAGTTCATCCATGCCAAGTGTAATCC		
23	down- <i>srfAC</i> _F	GGCATGGATGAACTATACAAATAAatgaagtgatgaaggaggagacagc	Amplification of downstream region of <i>srfAC</i>	
24	down_ <i>srfAC</i> _R	GGATCCCcaaacgtaaacagcaatacaaaccatttcaccc		
25	Vector_ <i>srfAD</i> _F	cattcggctgGGGGATCCTCTAGATTTCTATAGTGTACC	Amplification of pJOE6743.1 backbone for using as the vector	Construction of pMAV25
26	Vector_ <i>srfAD</i> _R	AGTAAAAGGCATGTCCCTATAGTGAGTCGTATTAGGCATGC		
27	up_ <i>srfAD</i> _F	CGACTCACTATAGGGACATGCCTTTTACTCATACTACGTCAACc	Amplification of end of <i>srfAB</i> gene with excluding the stop codon	
28	up- <i>srfAD</i> -R	CTTCTCCTTTACTAACCATcggttgaatgatcggtgctg		
29	eGFP- <i>srfAD</i> -F	gatcattcaaccgATGGTTAGTAAAGGAGAAGAAGCTTTTCACTG	Amplification of <i>mEGFP</i>	
30	eGFP- <i>srfAD</i> -R	ctgtccgcttttgaTTATTTGTATAGTTCATCCATGCCAAGTGTAATCC		
31	down- <i>srfAD</i> _F	GGATGAACTATACAAATAAatcaaaagcggacagcttcgg	Amplification of downstream region of <i>srfAD</i>	
32	down_ <i>srfAD</i> _R	GAGGATCCCCcagccgaatgaaaaataagatggatagcat		
33	mEGFP expression_F	gtttaactttaagaaggagatatacCCATGGTTAGTAAAGGAGAAGAAGCTTTTCACTG	Amplification of <i>mEGFP</i>	Construction of pMAV35
34	mEGFP expression_R	gtggtggtgctcgagtgcTTTGTATAGTTCATCCATGCCAAGTGTAATC		

Table S2. Strains and plasmids used in this study.

Strain or plasmid	Genotype or description	Reference
Strains		
<i>Escherichia coli</i>		
DH5a	$\Delta(argF-lac)169, \phi 80dlacZ58(M15), \Delta phoA8, glnX44(AS), deoR481, rfbC1, gyrA96(NalR), recA1, endA1, thiE1$ and <i>hsdR17</i>	(Song <i>et al.</i> , 2015)
BL21 (DE3) Gold	<i>F hsdSgal DE3</i>	
<i>Bacillus subtilis</i>		
3NA	<i>spo0A3</i> ;	(Reuß <i>et al.</i> , 2016)
BMV9	<i>spo0A3; $\Delta manPA$; sfp+</i> ;	(Vahidinasab <i>et al.</i> , 2020)
BMV25	<i>spo0A3; $\Delta manPA$; sfp+ ; srfAA-megfp</i> <i>mEGFP is Chromosomally integrated at the end of srfAA before the stop codon</i>	This study
BMV26	<i>spo0A3; $\Delta manPA$; sfp+ ; srfAB-megfp</i> <i>mEGFP is Chromosomally integrated at the end of srfAB before the stop codon</i>	This study
BMV27	<i>spo0A3; $\Delta manPA$; sfp+ ; srfAC-megfp</i> <i>mEGFP is Chromosomally integrated at the end of srfAC before the stop codon</i>	This study
BMV28	<i>spo0A3; $\Delta manPA$; sfp+ ; srfAD-megfp</i> <i>mEGFP is Chromosomally integrated at the end of srfAD before the stop codon</i>	This study
Plasmids		
pJOE6743.1	<i>ori_{pUC18}, bla, spc, manP, ter-'lacI-lacZa-ter</i>	(Wenzel and Altenbuchner, 2015)
pET-28a	Expression plasmid, <i>ori, kanR, lacI, his(7), tev site</i>	#20061, Addgene, Watertown, USA
pMAV22	pJOE6743.1 containing <i>srfAA'-megfp-'srfAB</i> (1000 bp up- and downstream of the C-terminal end of <i>srfAA</i> gene were used as flanking regions of the <i>megfp</i> gene)	(Denisov <i>et al.</i> , 2004) This study
pMAV23	pJOE6743.1 containing <i>srfAB'-megfp-'srfAC</i> (1000 bp up- and downstream of the C-terminal end of <i>srfAB</i> gene were used as flanking regions of the <i>megfp</i> gene)	This study
pMAV24	pJOE6743.1 containing <i>srfAC'-megfp-'srfAD</i> (1000 bp up- and downstream of the C-terminal end of <i>srfAC</i> gene were used as flanking regions of the <i>megfp</i> gene)	This study
pMAV25	pJOE6743.1 containing <i>srfAD'-megfp-'ycxA</i> (1000 bp up- and downstream of the C-terminal end of <i>srfAD</i> gene were used as flanking regions of the <i>megfp</i> gene)	This study
pMAV35	his-mEGFP expression plasmid using pET28a as expression vector	This study

References

- Denisov, I.G., Grinkova, Y.V., Lazarides, A.A., and Sligar, S.G. (2004) Directed self-assembly of monodisperse phospholipid bilayer Nanodiscs with controlled size. *Journal of the American Chemical Society* **126** (11): 3477–3487.
- Reuß, D.R., Thürmer, A., Daniel, R., Quax, W.J., and Stülke, J. (2016) Complete genome sequence of *Bacillus subtilis* subsp. *subtilis* strain Δ 6. *Genome announcements* **4** (4): 10.1128/genomea.00759-16.

- Song, Y., Lee, B.-R., Cho, S., Cho, Y.-B., Kim, S.-W., Kang, T.J., *et al.* (2015) Determination of single nucleotide variants in *Escherichia coli* DH5 α by using short-read sequencing. *FEMS Microbiology Letters* **362** (11): fnv073.
- Vahidinasab, M., Lilge, L., Reinfurt, A., Pfannstiel, J., Henkel, M., Morabbi Heravi, K., and Hausmann, R. (2020) Construction and description of a constitutive plipastatin mono-producing *Bacillus subtilis*. *Microbial Cell Factories* **19**: 1–12.
- Wenzel, M., and Altenbuchner, J. (2015) Development of a markerless gene deletion system for *Bacillus subtilis* based on the mannose phosphoenolpyruvate-dependent phosphotransferase system. *Microbiology* **161** (10): 1942–1949.