

Staphylococcal *saoABC* operon codes for a DNA-binding protein SaoC implicated in the response to nutrient deficit

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List S1. Occurrence of *saoABC* operon among staphylococcal species. The *saoABC* operon has been found in 47 out of 51 staphylococcal species. Negative species are underlined. It should be noted that in case of 4 species, in genomes of which we were unable to find the operon, the number of available genomic sequences was low (<10). Results for these species might be thus unreliable and in the future *saoABC* operon will likely be found in those species as well.

1. <i>S. agnetis</i>	14. <i>S. delphini</i>	27. <i>S. lentus</i>	40. <i>S. saccharolyticus</i>
2. <i>S. argensis</i>	15. <i>S. devriesei</i>	28. <i>S. lugdunensis</i>	41. <i>S. saprophyticus</i>
3. <i>S. argenteus</i>	16. <i>S. edaphicus</i>	29. <i>S. lutrae</i>	42. <i>S. schleiferi</i>
4. <i>S. arlettae</i>	17. <i>S. epidermidis</i>	30. <i>S. massiliensis</i>	43. <i>S. schweitzeri</i>
5. <i>S. aureus</i>	18. <i>S. equorum</i>	31. <i>S. microti</i>	44. <i>S. sciuri</i>
6. <i>S. auricularis</i>	19. <i>S. felis</i>	32. <i>S. muscae</i>	45. <i>S. simiae</i>
7. <i>S. capitis</i>	20. <i>S. fleurettii</i>	33. <i>S. nepalensis</i>	46. <i>S. simulans</i>
8. <i>S. caprae</i>	21. <i>S. gallinarum</i>	34. <i>S. pasteurii</i>	47. <i>S. stepanovicii</i>
9. <i>S. carnosus</i>	22. <i>S. haemolyticus</i>	35. <i>S. petrasii</i>	48. <i>S. succinus</i>
10. <i>S. chromogenes</i>	23. <i>S. hominis</i>	36. <i>S. pettenkoferi</i>	49. <i>S. vitulinus</i>
11. <i>S. cohnii</i>	24. <i>S. hyicus</i>	37. <i>S. piscifermentans</i>	50. <i>S. warneri</i>
12. <i>S. condimentii</i>	25. <i>S. intermedius</i>	38. <i>S. pseudintermedius</i>	51. <i>S. xylosus</i>
13. <i>S. cornubiensis</i>	26. <i>S. kloosii</i>	39. <i>S. rostri</i>	

Table S1. Primers used in the study. Restriction sites are double-underlined, start and stop codons as well as recombinase attachment sites (*attB1/attB2*) are in bold, ribosome-binding sites (RBSs) are underlined, group II L1.LtrB-ΔORF intron target sequences are in bold and underlined.

Name	Sequence	Use
pD-saoC-F	ATACATATGTTAACTAAAGAATTTGCACAAC G	pETDuet-saoC (NdeI/XhoI)
pD-saoC-R	AGACTCGAGTTACAGGTTGAATAAACG	
pC-saoB-F	CCGGTCGACTAACTAACTAATGGGGAGGTGT TCTTATGG	pCN51-saoB (Sall/BamHI)
pC-saoB-R	CGCGGATCCCTTAAAATGGTTTACGTAAATCC ATTTC	
pC-saoC-F	CCGGTCGACTAACTAACTAATGCAGAAAGG ACTTTAAAGTATG	pCN51-saoC (Sall/BamHI)
pC-saoC-F	CGCGGATCCCTTACAGGTTGAATAAACGTGCG	
pK-saoA-attB1-UF	GGGGACAAGTTTGTACAAAAAAGCAGGCT TGATGCCCATTTTGGAACTCTTTTC	pKOR-del-saoA (<i>attB1/SacII/attB2</i>)
pK-saoA-UR	AGGTCCCCGCGGAGTTGTGCCCCCTTTAAAA ATTG	
pK-saoA-DF	AGGTCCCCGCGGTTATCACACTGAATTCAAA ATGAGATTAC	
pK-saoA-attB2-DR	GGGGACCACTTTGTACAAGAAAGCTGGGT TTCTTCTAAATGTTGAACAATTTTACGGAC	
pK-saoC-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCT GTTCAAGAACATAACTCAGCATATG	pKOR-del-saoC (<i>attB1/SacII/attB2</i>)
pK-saoC-UR	AGGTCCCCGCGGACTTTAAAGTCCTTTCTGCA TATAC	
pK-saoC-DF	AGGTCCCCGCGGACTTGTGATTTGAACACAA ATTAAATAC	
pK-saoC-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGT ATCAGTGTAAGAAAAGCATTATGAAC	
pN-IBS1-saoB	AAAAAAGCTTATAATTATCCTTATATTACCAA ACCGTGCGCCCAGATAGGGTG	pNL9164-saoB
pN-EBS1-saoB	CAGATTGTACAAATGTGGTGATAACAGATAAG TCCAAACCTTTAACTTACCTTTCTTTGT	
pN-EBS2-saoB	TGAACGCAAGTTTCTAATTTTCGGTTAATAATC GATAGAGGAAAGTGCT	
pN-IBS1-saoC	AAAAAAGCTTATAATTATCCTTAGTAGACGA TTTAGTGCGCCCAGATAGGGTG	pNL9164-saoC
pN-EBS1-saoC	CAGATTGTACAAATGTGGTGATAACAGATAAG TCGATTACGTAACCTTACCTTTCTTTGT	
pN-EBS2-saoC	TGAACGCAAGTTTCTAATTTTCGATTCTACTC GATAGAGGAAAGTGCT	

pC-P-saoABC-F	GCTGCATGCATAACTGCTTAGGTAATCTT	pCN35-P-saoABC-gfpmut2-term
pC-P-saoABC-R	AGAGTCGACCAGGTTGAATAAACGTG	(PaeI/SalI/BamHI/EcoRI)
pC-gfpmut2-F	CAGGTCGACAGTAAAGGAGAAGAACTTTTC	
pC-gfpmut2-R	CGGGGATCCCTTATTTGTATAGTTCATCCATG	
pC-term-F	AGAGGATCCACTTGTGATTTGAACACAAATTT	
pC-term-R	CCTGAATTCAGGAATACACAGGAGCCA	
RT-saoA-F	TGTTAGTTGTAGCGGTTTCGTG	RT-qPCR
RT-saoA-R	GCTTTGGGACCTTTAGATGCT	
RT-saoB-F	AGATTTAACACAAGATAACGGCCTC	
RT-saoB-R	GTGTGACCATCATTAGTTGGATGAA	
RT-saoC-F	ATCAACAGACGCTAAGACGGA	
RT-saoC-R	GCTCAACGCTGGCTTTTTC	
RT-gyrB-F	CAACAATGAACCCTGAGCACC	
RT-gyrB-R	CGGTTTTCTACAACGTCACCC	
RT-23SRNA-F	CCGAAGCTGTGGATTGTCCT	
RT-23SRNA-R	GCTACTCACACCGGCATTCT	
saoA-prom-F	AAGTTTCATCAAATAAACTGTGACCATC	DNA pull-down
saoA-prom-R	TGTTCAACCATCTTCTCCTGTTAAC	
saoC-prom-F	TTCATCCAATAATGATGGTCACAC	
saoC-prom-R	TCTAATTCATCAAACGCTAGATCATAAC	
saoC-prom-fr1.2-F	ATTTATGCTTTATTAGAAGAAATGGATTACG	
saoC-prom-fr1-R	TGAAACGTCAGTCTTGCTTCATAC	
saoC-prom-fr2-R	TATACTGACGTTTGTCTACGGG	
saoC-prom-fr3-F	TTTAAACTATGTATGAAGCAAGACTGAC	
saoC-prom-fr3-R	CGTTGTGCAAATCTTTAGTTAACATAC	
saoC-rand-prom-F	CAAATAATAGGTACAAATCTAAAGACGG	Control for DNA pull-down
saoC-rand-prom-R1	CTCTCACTATTTTTATACGCCTTTTTATG	
saoC-rand-prom-R2	TGACTATAAGTAGCTAAAACATAATGTATTTG	

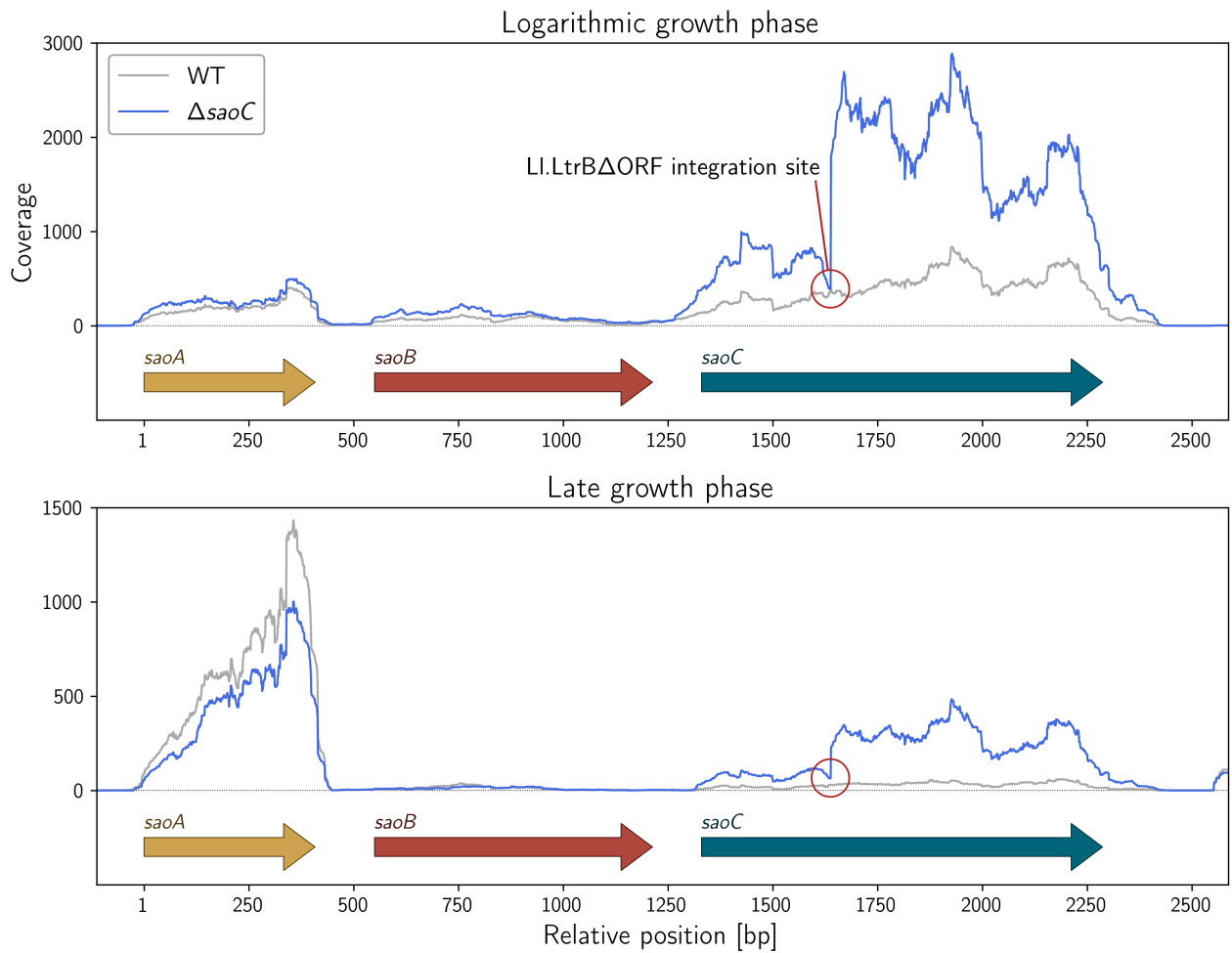


Figure S1. Coverage plots of reads mapped specifically to *saoABC* operon. The reads were derived from all RNA-Seq replicas either for the logarithmic (upper plot) or the late phase of growth (lower plot). In both cases clearly elevated coverage of *saoC* (disrupted by L1.LtrB Δ ORF intron) might be observed for Δ *saoC* mutant, which corroborate the claim that functional SaoC acts as a transcriptional repressor by binding to the conserved regulatory sequence upstream its own gene.

MbtS	1	-----MNLS-KQIKK---YRERDGYSQEYLAEKLYVSRQS-----	31
		:. :: :. .. . ::.. .. ::	
SaoC	1	MLTKEFAQRVELSEKQVRKIVQHLEERGYQ---LSKTEYRGREATDFKEE	47
MbtS	32	-----ISNWENDKSLPDIHN	46
		:. : : ...	
SaoC	48	DIELFKDIADKVKQTNSYDLAFDELEKEKDFLQVIVKN--DDKNLPTNQN	95
MbtS	47	LLMMCelfnvtLDDLvKGTIPFVPDIKAQRSlnLwTYVmlIFMTLAAILM	96
		:...:	
SaoC	96	VAQL-----	99
MbtS	97	GPLVVYWNWTWGVTVAILLGIGFYASMKIEDLK-----	129
		: :	
SaoC	100	-----VEDLRLEIQKMREERHLLGQMM	121
MbtS	130	-KVH-----	132
		:	
SaoC	122	NQVHQQQQELKELQNQLTSKIDSNSESLKAIQTSQEAIQEAQASQAKVLA	171
MbtS	133	-----KMDNYDRIVAFMNGKDPSEVQT-----	154
		. : . . .::..	
SaoC	172	ESTNKVEKNAVTEDEKADSKDSKVAGVNTSTDAKTDTKADNAGDGTtTKVD	221
MbtS	154	-----	154
SaoC	222	KEDQISATEAIEKASVEQSNGNAAETSNKEATVDAAEQHDAEQQVAEAHA	271
MbtS	155	-----TKARNTMTNALSIIIS-----VIGILSLI	177
	:. .:. :...:	
SaoC	272	EASKQATSNDsLEAKAENDSTASQSEMSEPKPQEEKKGFFARLFNL----	317
MbtS	178	IFLSVYLANkFL 189	
SaoC	317	----- 317	

Figure S2. Sequence alignment of MbtS, a transcription factor recently described for *S. aureus* [1], and SaoC. Sequence similarity between these two proteins is limited.

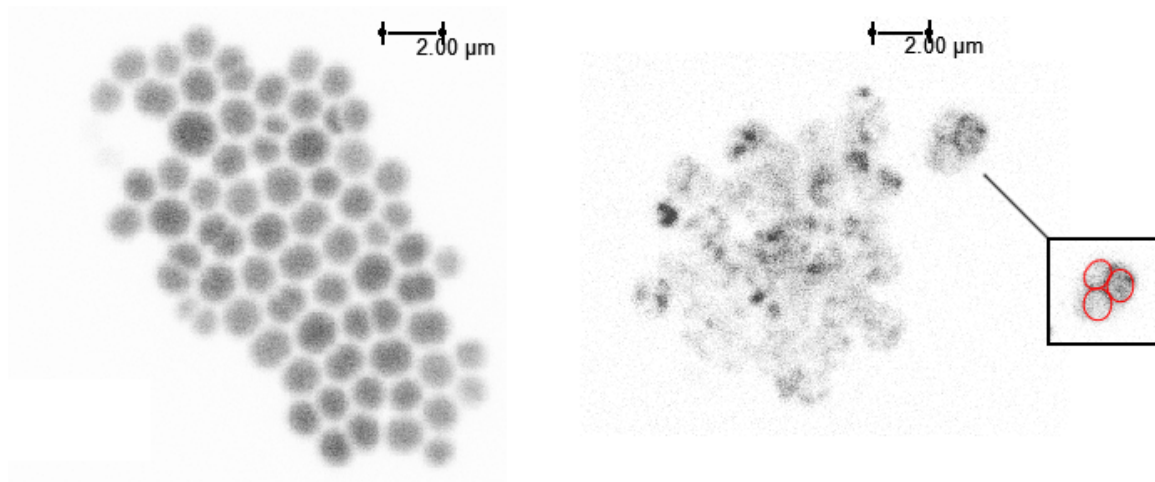


Figure S3. Confocal microscopy picture of *S. aureus* RN4220 transformed with pCN68 for free GFP expression (left) and pCN35-P-saoABC-gfpmut2-term carrying a copy of complete *saoABC* operon with *gfpmut-2* sequence insert for expression of SaoC-GFP fusion protein (right). In the latter case fluorescence signal forms ring-shaped halos, which indicates peripheral localisation of SaoC-GFP, likely at the inner surface of cell membrane.

CAAATAATAGGTACAAATCTAAAGACGGTAAGTGCTAGCCAACCTTGTATGATGCATTGT
AATAACTTCGTAAATAATAATAATTAACAAATACATTATGTTTTAGCTACTTATAGTCAC
CATAGCAAATAGTCGCTTCTAAGATAATCCACCAAATTTATTACTTATAGGTCACATTGT
AAGCTAGTCCGAGGAGTTTAAAAAGAGTGTTGCCATGATTTAGGGATTTGTAACCAGTCA
AAGGTCAACTTTCTTATATGGATTTGAACAAGAAATAGTGTTTTCTAGTTTTCTTCAATA
CGTCATAGTTGATGTGCTATCTCCAATTCACAGTATCTATTTCTGAATTGGACATGGCTG
AAGAAGGGAAAAGTTGAGGGTAGTCACAAAGTTTAAAGAATATTACATGGAATCGCCAGA
ATGAACTAAAATCATAGGCTTCCTTATGAAGTATCCCTCAGCGAACTGTCACACGGTAAC
TAAAGAGAAGAATGATCATAAAAAGGCGTATAAAAATAGTGAGAGT

Sequence S1. Shuffled *saoC* promoter sequence (saoC-rand-prom) used as a control for DNA pull-down experiments. Hybridisation sites for primers are underlined.

References

1. Yeo, W.S.; Anokwute, C.; Marcadis, P.; Levitan, M.; Ahmed, M.; Bae, Y.; Kim, K.; Kostrominova, T.; Liu, Q.; Bae, T. A Membrane-Bound Transcription Factor Is Proteolytically Regulated by the Aaa+ Protease Ftsh in *Staphylococcus Aureus*. *J. Bacteriol.* **2020**, *202*, e00019-20. <https://doi.org/10.1128/JB.00019-20>.