

1 **Supplementary information**

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3 **Supplementary Table 1. Bacteria and plasmids**

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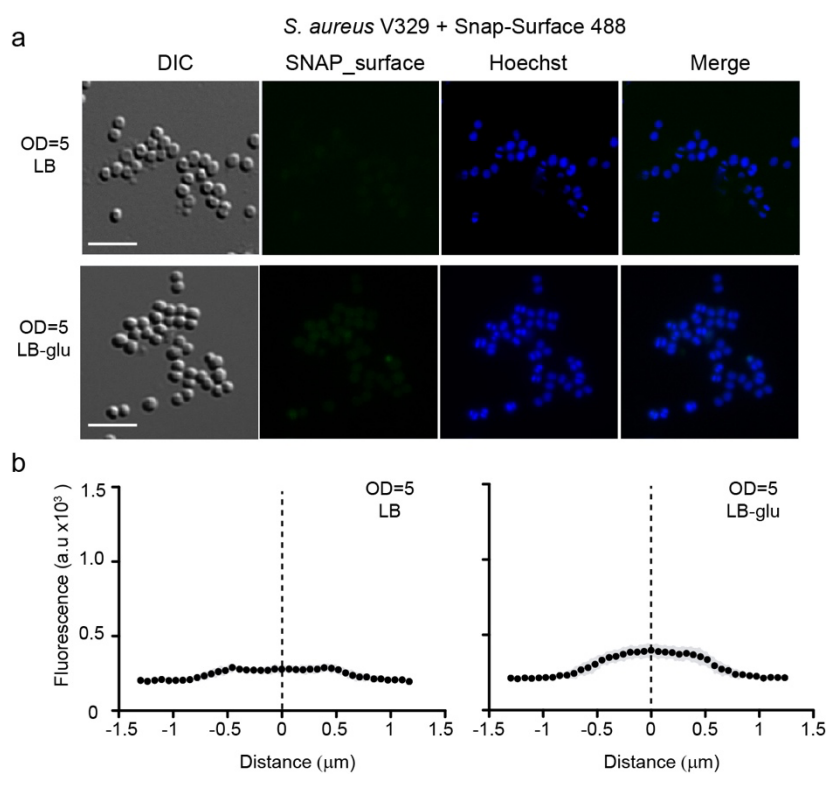
Strain name	Relevant characteristics	Source of reference
V329	Expresses Bap. Biofilm positive.	¹
V329 His	6xHis tag cloned at the end of B region of Bap	This study
V329 SpyTag	SpyTag cloned at the end of B region of Bap	This study
V329 Mefp3	Mefp3 gene cloned at the end of B region of Bap	This study
V329 MT1	MT1 gene cloned at the end of B region of Bap	This study
V329 SnapTag	SnapTag gene cloned at the end of B region of Bap	This study
V329 mCherry	mCherry gene cloned at the end of B region of Bap	This study
BL21 (DE3)	<i>F⁻ ompT hsdS_B(r_B⁻, m_B⁻) gal dcm lon λ(DE3 [lacI lacUV5-T7 gene1 ind1 sam7 nin5])</i>	Novagen
BL21(DE3)+pET46-ek/LIC:bap B:SpyTag	BL21 carrying pET46 for overexpression of Bap_B protein fused to SpyTag protein	This study
BL21(DE3)+pET46-ek/LIC:gfp:SpyCatcher	BL21 carrying pET46 for overexpression of GFP protein fused to SpyCatcher protein	This study
BL21(DE3)+pET46-ek/LIC:SpyCatcher	BL21 carrying pET46 for overexpression of SpyCatcher protein	This study
BL21(DE3)+pET46-ek/LIC:gfp	BL21 carrying pET46 for overexpression of GFP protein	This study
<i>E. faecalis</i> 23	esp-, non-biofilm forming	²
V329 Δ <i>bap</i>	V329 with deletion in <i>bap</i> gene	³
Plasmid name	Description	Source of reference
pJET1.2/blunt	Cloning vector	Termo Scientific
pET46-ek/LIC	Expression vector	Novagen
pSNAP-tag®(T7)-2	Expression vector encoding the SNAP-tag protein	New England Biolabs
pUA1108-GFPCatcher	Expression vector encoding <i>gfp-spycatcher</i> sequence under the expression of <i>Ptac</i> promoter	This study
pMAL-c2x-MT1	Plasmid used to amplify the metallothionein (MT1)	Adegene
pHRR	Plasmid used to amplify the <i>mcherry</i>	⁴
pET46-ek/LIC:bap B:SpyTag	pET46 for induction of Bap_B protein fused to SpyTag protein	This study
pET46-ek/LIC:gfp:SpyCatcher	pET46 for induction of GFP protein fused to SpyCatcher protein	This study
pET46-ek/LIC:SpyCatcher	pET46 for induction of SpyCatcher protein	This study
pET46-ek/LIC:gfp	pET46 for induction of GFP protein	This study
pMAD	<i>E. coli</i> - <i>S. aureus</i> shuttle vector with a thermosensitive origin of replication for Gram-positive bacteria. The vector contains the <i>bgaB</i>	⁵
pMAD:6xHis	pMAD for integrating 6xHis tag in the chromosome	This study
pMAD:SpyTag	pMAD for integrating SpyTag tag in the chromosome	This study
pMAD:Mefp3	pMAD for integrating Mefp3 tag in the chromosome	This study
pMAD:MT1	pMAD for integrating MT1 tag in the chromosome	This study
pMAD:SnapTag	pMAD for integrating SnapTag tag in the chromosome	This study
pMAD:mCherry	pMAD for integrating mCherry tag in the chromosome	This study

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1 Supplementary Table 2. Oligonucleotides and plasmids used in this study

Construction	Primer name	Sequence 5'-3'
Bap-Snap	Bap_SNAP_A_EcoRI	5'-GAATTCGAAACTGTAGGCGTTAGAGT
	Bap_SNAP_B	5'-GGTGGTGCCTTCATTTTCGAGTCTTTGTCCATATTTACAGTTGCTGTACCAAC
	Bap_SNAP_C	5'-GAGGGCCACAGACTGGGCAAGCCTGGGCTGGGTTTCAGATGGTACATTCTCAGTG
	Bap_SNAP_D_BamHI	5'-GGATCCTGTATTTACTTTCATCTAATGTTGG
	SNAPtag_A	5'-TTGGTACAGCAACTGTAAATATGGACAAAGATTGCGAAATGA
	SNAPtag_A	5'-ACACTGAGAATGTACCATCTGATCCCAGACCCGGTTTACCCAG
Bap-HIS	BapB_B_HIS_rev2	5'-GTGGTGATGGTGATGATGATTTACAGTTGCTGTACCAACTGTTGTACCCT
	BapB_C_HIS_fw	5'-CATCATCACCATCACCCTCAGATGGTACATTCTCAG
Bap-Spy	Bap_Spy_B	5'-CTTCGTCGGCTTGTAGGCGTCCACCATCACGATGTGGGCATTTACAGTTGCTGTACCAAC
	Bap_Spy_C	5'-GCCACATCGTGATGGTGGACGCCTACAAGCCGACGAAGTCAGATGGTACATTCTCAGT
	Spy_tag_fw	5'-GGGCCAGCCGGTACCGCCACATCGTGATGGTGGACGCCTACAAGCCGACGAAG
	Spy_tag_rv	5'-GGGCCAGCCGGTACCCCTTCGTCGGCTTGTAGGCGTCCACCATCACGATGTGGGC
Bap-Mef3	Bap-Mefp3-B	5'-CGGGCCATAGTAATCCGCATTTACAGTTGCTGTACCAAC
	Bap-Mefp3-C	5'-AGACGCGGCAAATACTGGTTCAGATGGTACATTCTCAGTG
	Mefp3-Fw	5'-GCGGATTACTATGGCCC
	Mefp3-Rv	5'-CCAGTATTTGCCGCGT
Bap-mCherry	Bap-mCherry-B	5'-TCTTCTTCACCTTTACTAGTATTTACAGTTGCTGTACCAAC
	Bap-mCherry-C	5'-GGTATGGATGAATTATACAAAGATGGTACATTCTCAGTGTCA
	mCherry-pHRR-Fw	5'-ACTAGTAAAGGTGAAGAAGATA
	mCherry-pHRR-Rv	5'-TTTGTATAATTCATCCATACCAC
Bap-MT1	Bap_Oro_B	5'-GGAGCAGTTGGGGTCCATATTTACAGTTGCTGTACCAAC
	Bap_Oro_C	5'-GTGCACGTGCTGTGCCTCAGATGGTACATTCTCAGT
	Oro_A	5'-TACAGCAACTGTAAATATGGACCCCAACTGCTCC
	Oro_B	5'-ACACTGAGAATGTACCATCTGAGGCACAGCACGTGCAC
rBapB_Spy	Bap-LIC-Fw	5'-GACGACGACAAGATGCAAAAATCTTTAGGTTACACAGATAATTATAC
	Bap-LIC-Rv	5'-GAGGAGAAGCCCGGTTCTTCGTCGGCTTGTAGGCGTCCACCATCACGATGTGGGCGCCAGAACCACCG TGCCATTTACAGTTGCTGTACCAACTGTTGTAC
Catcher-Gfp	GFP_Ek_LIC_Fw	5'-GACGACGACAAGATGAGTAAAGGAGAAGAAGAACTTTTC
	Catcher_EK-LIC_Rv	5'-GAGGAGAAGCCCGGTTAAATATGAGCGTCACCTTTAG
Gfp	GFP_Ek_LIC_Fw	5'-GACGACGACAAGATGAGTAAAGGAGAAGAAGAACTTTTC
	GFP_Ek_LIC_Rv	5'-GAGGAGAAGCCCGGTTATTTGTATAGTTCATCCATGCC

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Supplementary Figure 1: Negative control for SNAP fluorescence. a) *S. aureus* expressing the Bap without SNAP-tag was grown in LB and LB-glu until stationary phase (OD₆₀₀=5). Cells were labelled with SNAP-surface 488 substrate and Hoechst. The fluorescence of SNAP-surface 488 and Hoechst, the combination of both signals (merge panels) and the differential interference contrast (DIC) images are shown. Scale bar of panels represents 5 μm. b) The fluorescence intensity was determined using the Intensity profile plugin of the Icy-software. Graphs correspond to the mean of the intensity profiles of cross-sections cells (n=20). Gray shadow corresponds to the standard deviation of the mean.

1 **Supplementary references.**

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