Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Material for

Title: Matrix composition determines dimensions of *Bacillus subtilis* NCIB 3610 biofilm colonies grown on LB agar.

Running title: Dimensions of *Bacillus subtilis* biofilm colonies

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Supplementary Tables:

Supplementary S1 Table: Growth rates (GR) of strains grown in liquid media. Growth rates have been determined as described in Methods.

Bacterial strain	**GR (1/min) ± sdev**
B-1	0,037 ± 0,002
NCIB 3610	0,037 ± 0,006
CA017 *(tasA::kan)*	0,038 ± 0,005
N24 (*bslA::cat*)	0,035 ± 0,005
ZK3660 (*epsA-O::tet*)	0,038 ± 0,005
bslA/tasA *(bslA::cat, tasA::kan)*	0,036 ± 0,004
BD630	0,032 ± 0,001

Supplementary S2 Table: Fit parameters for data presented in S5 Fig A-D. Fitting was performed as described in **Methods**. Predicted values for the *bslA/tasA* double deletion mutant are a result of only considering the component contribution factors (**S3 Table**) and do not include fitting results of the *bslA/tasA* mutant.

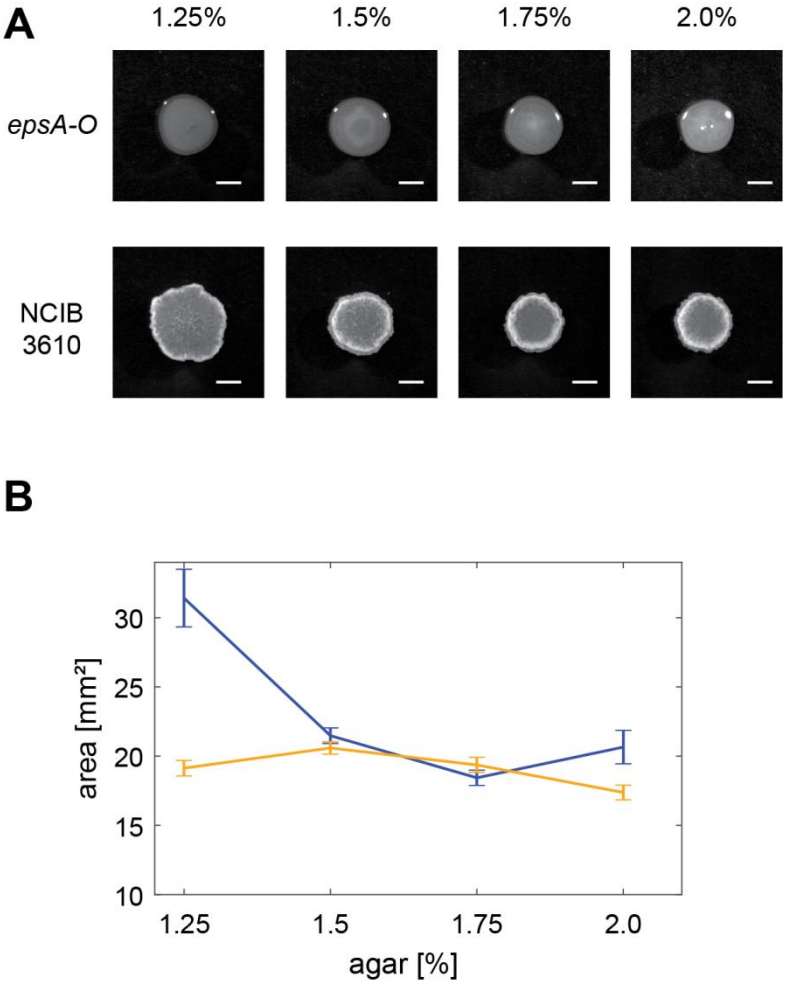
	Strain							
Area Fit parameter	B-1	BD630	NCIB 3610	*bslA*	*epsA-O*	*tasA*	*bslA/tasA*	predicted *bslA*/*tasA*
base [mm²]	6.50	6.50	6.50	6.50	6.50	6.50	6.50	
P0 [mm²]	0.75	1.20	0.31	0.26	0.53	1.04	0.58	
k [mm²]	17.79	20.18	10.85	18.97	16.17	10.13	13.85	17.72
r [1/h]	0.48	0.26	0.45	0.35	0.23	0.29	0.21	
Estimated final area [mm²]	24.29	26.68	17.35	25.47	22.67	16.63	20.36	
adjusted R^2	0.96	1.00	1.00	1.00	1.00	0.98	1.00	
Number of observations	36	14	27	25	34	32	42	

	Strain							
Height Fit parameter	B-1	BD630	NCIB 3610	*bslA*	*epsA-O*	*tasA*	*bslA/tasA*	predicted *bslA*/*tasA*
base [µm]	3.99	3.99	3.99	3.99	3.99	3.99	3.95	
P0 [µm]	0.33	0.43	5.55	2.53	0.48	4.35	0.63	
k [µm]	395.61	66.81	157.15	107.99	98.04	195.97	131.88	134.60
r [1/h]	0.93	0.73	0.34	0.46	1.17	0.44	0.80	
Estimated final height [µm]	399.60	70.80	161.13	111.98	102.03	199.95	135.83	
adjusted R^2	0.98	0.99	0.98	0.97	0.99	0.98	0.99	
Number of observations	14	14	14	21	14	14	16	

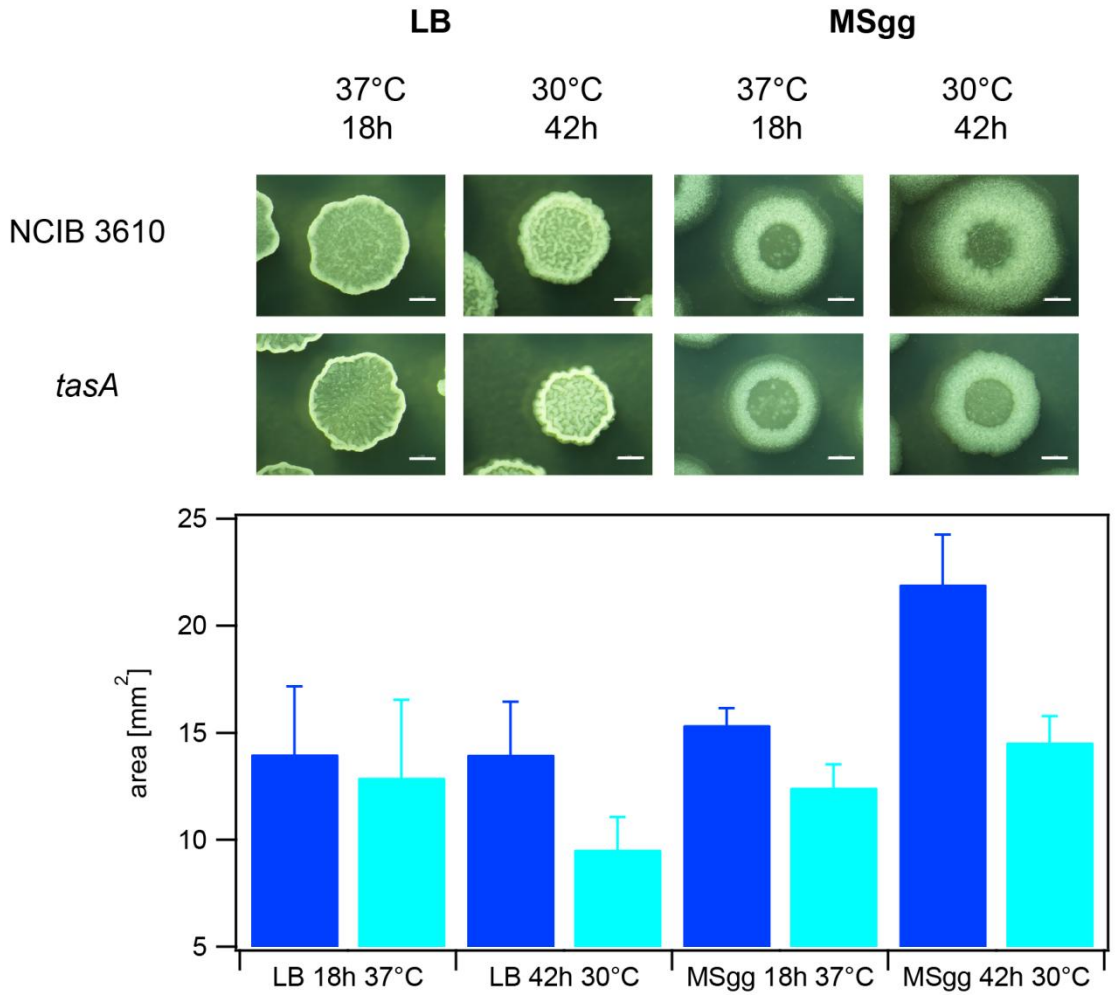
Supplementary S3 Table: Contribution of the different matrix components to growth parameters as obtained by theoretical modeling. The error is given as the standard deviation.

Observable	β_0	β_{BslA}	β_{EpsA}	β_{TasA}
Area [mm²]	15.10 ± 3.41	0.57 ± 0.07	1.17 ± 0.26	1.07 ± 0.11
Height [µm]	122.21 ± 32.54	1.46 ± 0.29	1.10 ± 0.10	0.80 ± 0.21
Roughness Sq	0.08 ± 2.01	28.57 ± 62.38	2.39 ± 59.22	2.91 ± 2.55

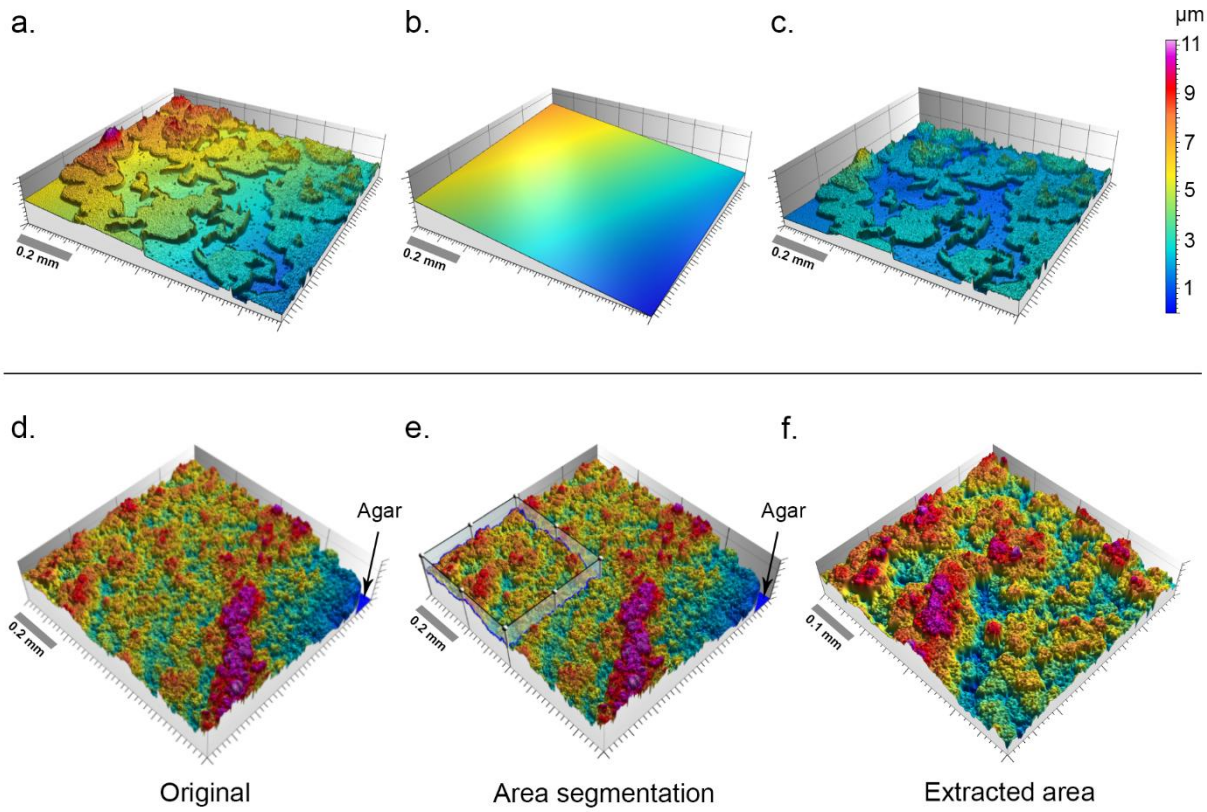
Supplementary Figures:



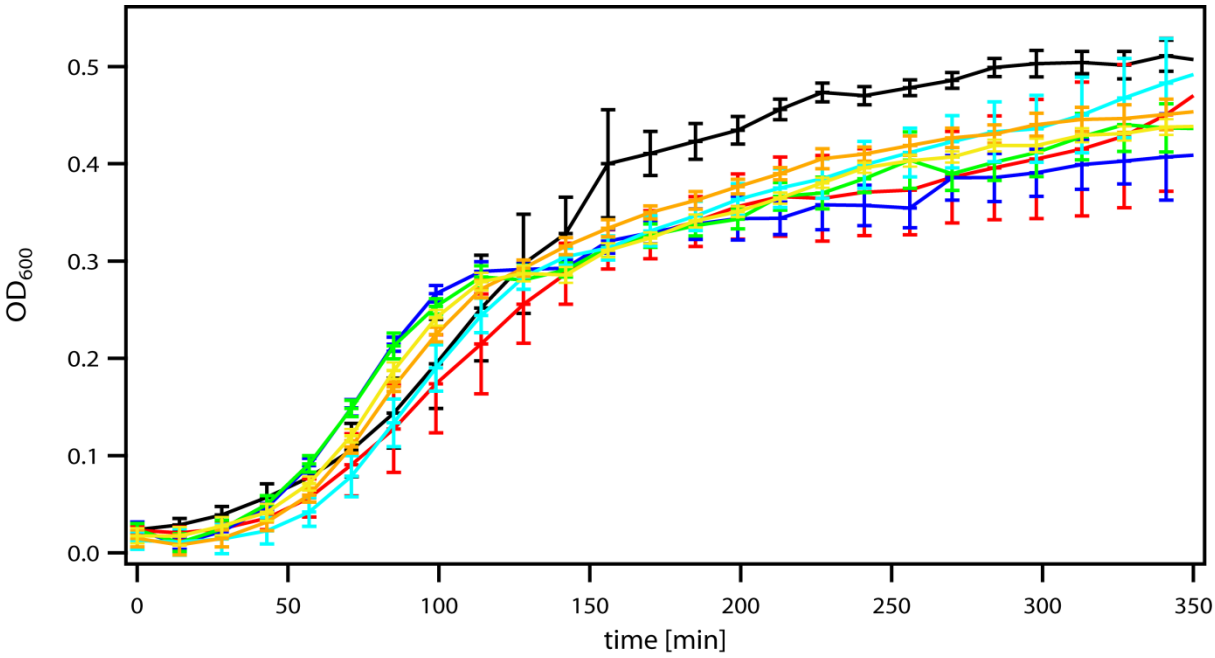
Supplementary S1 Figure: Final area occupied by biofilm colonies formed by the *epsA-O* mutant in comparison to biofilm colonies formed by the NCIB 3610 wild type strain on solid surfaces with different agar concentrations after 21 h. A) Exemplary microscopic images of the strains grown on solid surfaces with different agar concentrations. Scale bar: 2 mm. B) Area growth versus agar concentration. NCIB 3610 data shown in blue, *epsA-O* data shown in orange. For the *epsA-O* biofilm colony area stays constant for all agar concentrations tested.



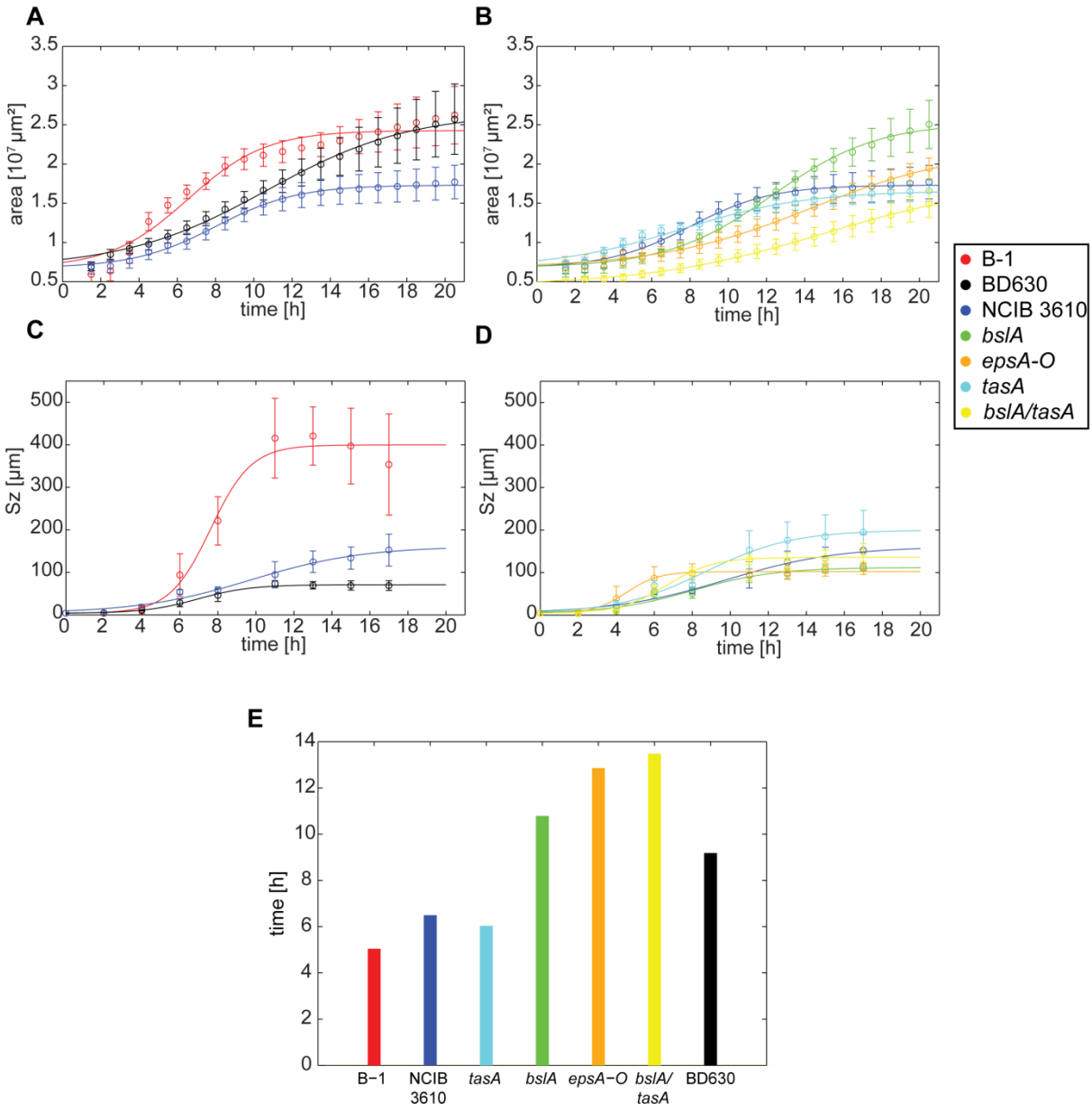
Supplementary S2 Figure: Growth conditions affect the size of biofilm colonies. If grown at 30°C the biofilm colony of the *tasA* mutant covers a smaller area as the biofilm colony of the wild-type NCIB 3610 on both LB and MSgg medium. At 37°C the biofilm colonies of both strains are similar in size. Biofilm colony morphology differs considerably for the two different media types. Scale bar represents 1 mm.



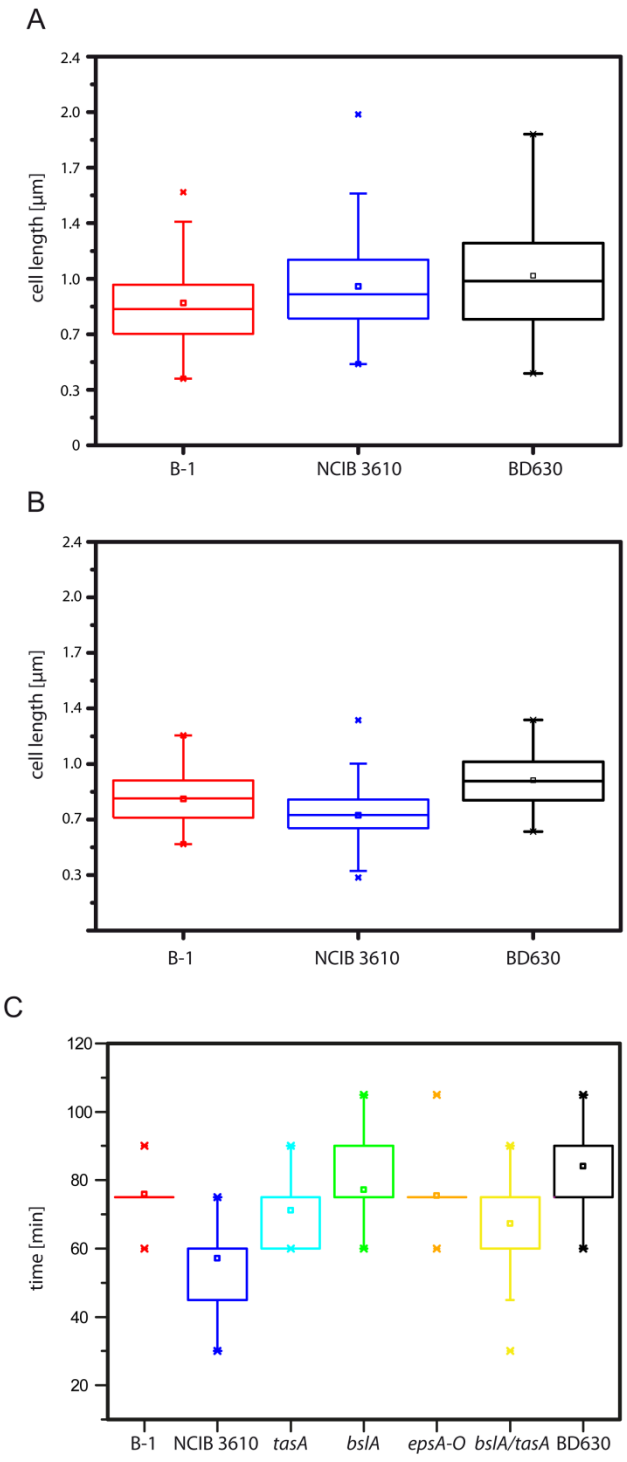
Supplementary S3 Figure: Correction of data obtained by light profilometry. For the calculation of the parameters ***Sq*** and ***Sz*** (**Methods**), a correction method was applied to images obtained for early stages of biofilm formation. a-c: the agar layer (b) was subtracted from the original image (a) using a linear correction which removes the tilt of the surface. The resulting image (c) shows a surface profile corresponding to the biofilm colony only. d-f: for the calculation of ***Sq*** at growth times later than 5 h, only data from the image quadrant opposite of the agar border was analyzed. d) original image, e) original image indicating the area to be segmented, f) extracted area. a-e: scale bar represents 0.2 mm. f) scale bar represents 0.1 mm.



Supplementary S4 Figure: Bacterial growth in solution. Data represent averaged growth curves of strains NCIB 3610 (dark blue), *tasA (*light blue), *bslA* (green), *epsA-O* (orange), B-1 (red), *bslA/ tasA* (yellow) and BD630 (black). Bacterial growth rates were obtained as described in **Methods**.



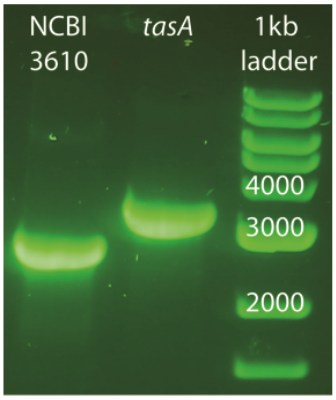
Supplementary S5 Figure: Sigmoidal fitting of experimentally obtained area and height growth curves. The corresponding fit parameters are given in **S2 Table.** A: Fitting of macro-colony biofilm growth as presented in **Fig 2c**. B: Fitting of macro-colony biofilm growth as presented in **Fig 3c**. Fitting of vertical growth as presented in **Fig 2d**. D: Fitting of vertical growth as presented in **Fig 3d.** E: Inflection points of the sigmoidal fits applied to area growth curves (**Fig 2c** and **Fig 3c)** for the respective wild-type and mutant strains.



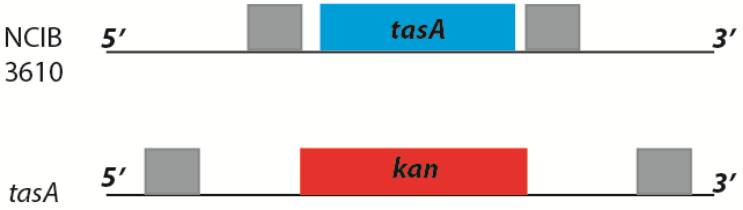
Supplementary S6 Figure: Individual cell size and time-point of initiation of three-dimensional growth. Boxplots show the distribution of the respective observable. The stars represent the highest and the lowest value observed. The box itself represents the interquartile range (25% - 75%) of the data. The line dividing the box is the median, the small square represents the mean. The bars go to the 5 percentile and the 95 percentile respectively. A: Initial size of single cells. B: Size of individual bacteria 60 min after inoculation (micro-colony area growth). C: Time-point of initiation of growth into the third dimension (micro-colony area growth). Because

this distribution is discrete due to the fact that the images are spaced 15 min apart, some boxes are distorted.

A



B



C

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D

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Supplementary S7 Figure: Confirmation of the deletion of the TasA protein in strain CA017. A: PCR analysis showing the expected 2785 bp PCR product for the TasA protein present in the wild-type NCIB 3610 and the 3478 bp PCR product carrying the kanamycin resistance gene instead of the TasA protein in the *tasA* deletion mutant. PCR was performed using the primer pair

AAGGATGGGAACGTGATCGAAAAAG/ AGGCGGTTAACAGGTGGAAGAATGA binding ~1000 base pairs (bps) before and behind the *tasA* gene, respectively. B: Schematic of the genes present in strains NCIB 3610 and CA017 (*tasA*). The *tasA* gene is given in blue. Grey boxes indicate sequences that are identical in both strains and correspond to the 100-200 bps before and after the *tasA* gene in the wild-type strain, respectively. The kanamycin resistance gene is shown in red in the CA017 strain. Sequencing was performed using the primer pair CATATGCCGGCTATATGC/ CGAATACGATGGTCAATTAG binding to sequences ~200 bps before and after the *tasA* gene, respectively. C) Sequence of strain NCIB 3610 including the *tasA* gene (blue). D) Sequence of strain CA017 including the kanamycin resistance gene (red) demonstrating the absence of the *tasA* gene. Boxes in grey show sequences that are identical in both strains and correspond to the 100-200 bps before and after the *tasA* gene in the wild-type strain.