Supporting Information

for

Patterned Biofilm Formation Reveals a Mechanism for Structural Heterogeneity in Bacterial Biofilms

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Table S1. List of *E. coli* strains and plasmids used in this study.

	Genotype	Characteristics	Source/References
***E. coli* Strains**			
RP437	Wild type [*thr-1*(Am) *leu*B6 *his-4 metF159*(Am) *eda*-50 *rpsL*1356 *thi*-1 *ara*-14 *mtl*-1 *xyl*-5 *tonA*31 *tsx*-78 *lacY*1 F⁻]	Wild type strain for biofilm study	[1]
RP3087	RP437 *(motB)580*	Motility mutant (point mutation in *motB*)	[2]
RP4979	RP437 *eda⁺Δm43-10(cheY)*	Chemotaxis mutant	[3, 4]
RP3525	RP437 Δ*tap-365-4* Δ*(lac)U169 thr⁺ leu⁺*	Chemotaxis mutant	[5, 6]
KX1485	RP437 *luxS::*Cmʳ	Quorum sensing mutant, unable to synthesize AI-2	[7, 8]
BL-19	RP437 Δ*motB*	Motility mutant (deletion mutation of *motB*)	[2]
Plasmids			
pRSH109	Ampʳ, *gfp*	Label cells with constitutive GFP	This study
pDsRed	Ampʳ, *rfp*	Label cells with constitutive RFP	Life Technologies Inc., Carlsbad, CA, USA
pRHG01	*luxS⁺*, Ampʳ	pCR2.1®-TOPO® with *luxS* gene controlled by its native promoter	This study

Table S2. Pearson correlation coefficients between Cell $\underline{C}$luster $\underline{I}$nteraction $\underline{I}$ndex (CII) and pattern size.

Distance (D, µm)	Pattern Size (W, µm)	Correlation Coefficient (r)
10	≤ 20 µm × 20 µm	-0.45[**]
	>20 µm × 20 µm	0.93[**]
15	≤ 20 µm × 20 µm	-0.23[*]
	>20 µm × 20 µm	0.94[**]
20	≤ 20 µm × 20 µm	-0.20
	>20 µm × 20 µm	0.93[**]
30	≤ 20 µm × 20 µm	-0.20
	>20 µm × 20 µm	0.93[**]
40	≤ 20 µm × 20 µm	-0.25[*]
	>20 µm × 20 µm	0.92[**]
50	≤ 20 µm × 20 µm	-0.47[**]
	>20 µm × 20 µm	0.95[**]

* $P < 0.05$.
** $P < 0.0001$.

Table S3. Biofilm formation of *E coli* RP437 and its isogenic mutants on patterned surfaces.

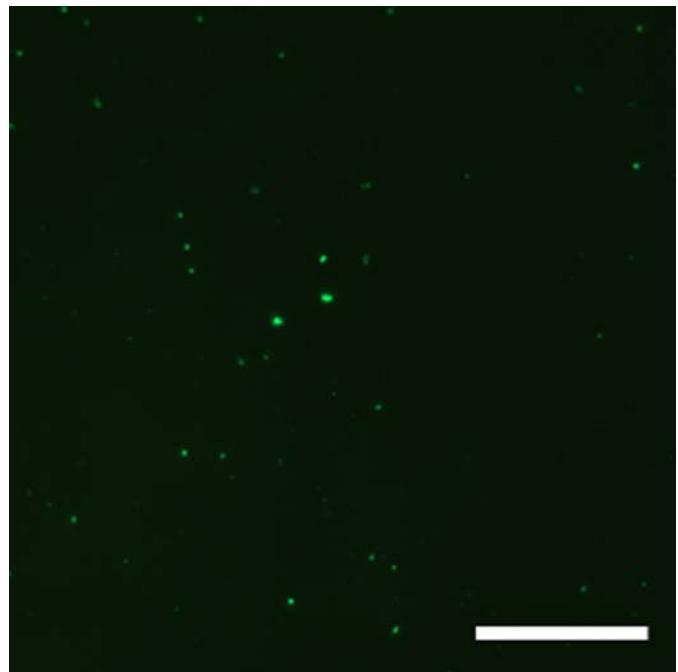| Genotype	Attachment on patterns	Interaction among cell clusters
Wild-type	Fully covered patterns	Normal interaction
ΔcheY	Barely covered	No interaction
Δtap	Barely covered	No interaction
motB point mutant	Relatively normal cell clusters (slightly less coverage than the wild type)	Significantly reduced interaction
ΔmotB	Barely covered	No interaction
ΔluxS	Relatively normal cell clusters (slightly less coverage than the wild type)	Significantly reduced interaction

Figure S1. Attachement of *E. coli* BL-19 (RP437 *ΔmotB*) on a surface with 20 µm × 20 µm SAM patterns after 24 h of incubation. Bar = 50 µm.

References:

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