**Table S1. Bacterial strains and plasmids used in this study.**

|  |  |  |
| --- | --- | --- |
| **Strains/plasmids** | **Relevant Features** | **Reference** |
| ***E. coli*** |  |  |
| S17-*pir* | Wild Type | de Lorenzo and Timmis, 1994 |
|  |  |  |
| ***V. cholerae*** |  |  |
| C6706*str*2 | El Tor Wild Type | Thelin and Taylor, 1996 |
| JY283 | *vpvC*W240R *pomA* (denoted WT) | Yan et al., 2017 |
| JY285 | *vpvC*W240R *pomA**bap1**rbmC* | Yan et al., 2017 |
| JY286 | *vpvC*W240R *pomA**rbmA**bap1**rbmC* | Yan et al., 2017 |
| JY287 | *vpvC*W240R *pomA**vpsL* | Yan et al., 2017 |
| JY370 | *vpvC*W240R*pomA lacZ*:Ptac-*mKate2*:*lacZ* | Yan et al., 2017 |
| JY376 | *vpvC*W240R*pomA* *vpsL lacZ*:Ptac-*mKate2*:*lacZ* | Yan et al., 2017 |
| JY395 | *vpvC*W240R*pomA* *bap1**rbmC lacZ*:Ptac-*mKate2*:*lacZ* | This study |
|  |  |  |
| **Plasmid** |  |  |
| pKAS32 | Suicide vector, AmpR SmS | Skorupski et al., 1996 |
| pNUT144 | Suicide vector, AmpR KanR SmS | Drescher et al., 2014 |
| pNUT157 | pNUT144 *vpvC*W240R | Drescher et al., 2014 |
| pCMW112 | pKAS32 *vpsL* | Hammer and Bassler, 2003 |
| pCN004 | pKAS32 *lacZ*:Ptac-*mKate2*:*lacZ* | Nadell and Bassler, 2011 |
| pCN007 | pKAS32 *rbmA* | Nadell et al., 2015 |
| pCN008 | pKAS32 *rbmC* | Nadell et al., 2015 |
| pCN009 | pKAS32 *bap1* | Yan et al., 2016 |
| pCDN010 | pKAS32 *pomA* | Nadell et al., 2015 |
|  |  |  |

**References for Table S1**

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Nadell CD, Bassler BL. 2011. A fitness trade-off between local competition and dispersal in *Vibrio cholerae* biofilms. *Proc Natl Acad Sci USA* **108**:14181-14185. doi:10.1073/pnas.1111147108

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Skorupski K, Taylor RK. 1996. Positive selection vectors for allelic exchange. *Gene* **169**:47–52. doi:10.1016/0378-1119(95)00793-8

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Yan J, Sharo AG, Stone HA, Wingreen NS, Bassler BL. 2016. *Vibrio cholerae* biofilm growth program and architecture revealed by single-cell live imaging. *Proc Natl Acad Sci USA* **113**:E5337-E5343. doi:10.1073/pnas.1611494113

**Table S2: Key Resource table**

|  |  |  |
| --- | --- | --- |
| **Reagents** | | |
| LB broth, Miller | ThermoFisher | Cat# BP1426-2 |
| Bacto agar | VWR | Cat# 214030 |
| O.C.T. agent | Tissue-Tek, Sakura | Cat# 4583 |
| Silicone oil, 5 cSt | Sigma Aldrich | Cat# 317667 |
| Glass beads, acid washed, 425 – 600 m diameter | Sigma Aldrich | Cat# G8772 |
| MP Biomedicals™ Roll & Grow™ Plating Beads, 4 mm in diameter | ThermoFisher | Cat# MP115000550 |
| BD PrecisionGlide™ needles (0.6 mm×2.5 mm) | Sigma Aldrich | Cat# Z118044 |
| EMD Millipore, 25 mm in diameter | Sigma Aldrich | Cat# VSWP02500 |
| Sytox Green Nucleic Acid Stain | ThermoFisher | Cat# S7020 |
| Wheat Germ Agglutinin Sampler Kit | ThermoFisher | Cat# W7024 |
| Higgins Black India Ink |  |  |
| **Software and Algorithms** | | |
| MATLAB and Image Processing Toolkit | Mathworks, 2015 | https://www.mathworks.com/products/matlab.html |
| PRISM version 6.07 | GraphPad, 2015 | https://www.graphpad.com/scientific-software/prism/ |
| Image composite editor version 2.0.3 | Microsoft, 2015 | https://www.microsoft.com/en-us/research/project/image-composite-editor/ |
| Gmsh version 3.0.6 | (Geuzaine and Remacle, 2009) | https://gmsh.info |
| Paraview version 5.5.0 | (Ahrens et al., 2005) | https://www.paraview.org/ |
| FEniCS version 2017.2.0 | (Alnæs et al., 2015) | https://fenicsproject.org/ |
| DigiCamControl software version 2.0.72.0 | DigiCamControl, 2015 | http://digicamcontrol.com/ |
| Leica Map Start version 7.4.8051 | Leica, 2017 | https://www.leica-microsystems.com/products/microscope-software/details/product/leica-map/ |
| ImageJ and freehand line selection tool | NIH | https://imagej.nih.gov/ij/ |
| RheoPlus version 3.40 | Anton Paar, 2008 | N/A |
| **Instruments** | | |
| Physica MCR 301 shear rheometer | Anton Paar, 2008 | N/A |
| Nikon D3300 SLR digital camera with DX Zoom-Nikkor 18-55 mm lens | Amazon | https://www.amazon.com/Nikon-1532-18-55mm-3-5-5-6G-Focus-S/dp/B00HQ4W1QE/ref=sr\_1\_3?ie=UTF8&qid=1492108083&sr=8-3&keywords=D3300&th=1 |
| Huion L4S light box | Amazon | https://www.amazon.com/Huion-L4S-Light-Box-Illumination/dp/B00J0UUHPO |
| Sigma 105 mm macro lens for Nikon DSLR camera | Amazon | https://www.amazon.com/Sigma-258306-105mm-Macro-Camera/dp/B0058NYW3K/ref=sr\_1\_sc\_3?ie=UTF8&qid=1485483491&sr=8-3-spell&keywords=sigma+macroles |
| Leica stereoscope model M205 FA | Leica | N/A |
| Leica DCM 3D micro-optical system | Leica | https://www.leica-microsystems.com/products/light-microscopes/upright-microscopes/details/product/leica-dcm-3d/ |
| VR3200 wide-area 3D measurement system | Keyence | https://www.keyence.com/products/measure-sys/3d-measure/vr-3000/models/vr-3200/index.jsp |
| FEI XL 30 FEG-SEM | FEI | https://iac.princeton.edu/equipment.html |
| Millrock Technology, BT85A-A | Millrock | https://www.millrocktech.com/ |
| VCR IBS/TM200S ion beam sputterer | VCR | https://iac.princeton.edu/equipment.html |

**Table S3: Summary of biomaterial parameters for *V. cholerae* biofilms**

Biomaterial parameters for *V. cholerae* biofilms grown on different concentration agar substrates (denoted Agar conc.). *G*s and *G*f correspond to the shear modulus of the substrate and the biofilm, respectively. *h* and *h*r correspond to the total biofilm thickness and the thickness of the residual layer, respectively. *λ* corresponds to the wavelength of the wrinkles/blisters extracted from time-lapse images.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Agar conc.** | ***G*s (kPa)** | ***G*f (kPa)** | ***h* (μm)** | ***h*r (μm)** | ***λ* (μm)** |
| 0.4% | 0.14 ± 0.01 | 0.8 ± 0.1 | 57 ± 3 | 2.1 ± 0.9 | 506 ± 11 |
| 0.5% | 0.33 ± 0.04 | 1.1 ± 0.2 | 60 ± 4 | 1.8 ± 0.4 | 445 ± 12 |
| 0.6% | 0.51 ± 0.08 | 1.1 ± 0.1 | 64 ± 6 | 5.8 ± 1.5 | 422 ± 20 |
| 0.7% | 1.4 ± 0.2 | 1.5 ± 0.3 | 68 ± 1 | 7.7 ± 0.7 | 380 ± 20 |
| 0.8% | 1.9 ± 0.2 | 1.2 ± 0.2 | 70 ± 6 | 11 ± 2 | 380 ± 13 |
| 1.0% | 3.8 ± 1.1 | 1.1 ± 0.1 | 96 ± 8 | 30 ± 9 | 334 ± 17 |
| 1.5% | 12 ± 7 | 1.4 ± 0.03 | 118 ± 10 | 48 ± 0.3 | 308 ± 28 |
| 3.0% | 67 ± 10 | 2.4 ± 0.1 | 118 ± 3 | 48 ± 7 | 279 ± 10 |