Co-Expression of Type 1 Fimbriae and Flagella in *Escherichia coli*: Consequences for Adhesion at Interfaces

Udayanidhi Ramesh Kumar [a], Nam T. Nguyen [a], Narendra K. Dewangan [a], Sayed Golam Mohiuddin [a], Mehmet A. Orman [a], Patrick C. Cirino *[a], and Jacinta C. Conrad *[a]

*Corresponding authors

[a] Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX 77204, USA
E-mail: pccirino@uh.edu, jcconrad@uh.edu

ELECTRONIC SUPPLEMENTARY INFORMATION

Strain construction:

Strain MG1655Δ*fimA* was constructed by deleting *fimA* from *E. coli* K-12 MG1655 using the λ Red recombinase-based method, as described by Datsenko and Wanner.[1] The following primers and plasmids were used to generate the knockout cassette: *fimA*P1 (forward), *fimA*P2 (forward), *fimA*P1 (reverse), *fimA*P2 (reverse) and plasmid pKD13. The kanamycin resistance gene was removed using plasmid pCP20.

Plasmid construction:

Plasmid pPCC2000 ("p(blank)") was derived from vector pPCC1322[2], carrying the *lac* repressor gene (*lacI)* and apramycin resistance gene (*aac).*

Plasmid pPCC2208 was constructed by linearizing plasmid pPCC1401 with XbaI restriction enzyme (New England Biolabs). Next, a gene construct containing P*tac-fliC-flhD-flhC* was assembled by amplifying *fliC* and *flhDC* from *E. coli* MG1655 genomic DNA, and subcloning these into lab plasmid pPCC2206. P*tac-fliC-flhD-flhC* was then PCR-amplified using Phusion Polymerase (New England Biolabs) and primers p2207P1 (forward) and p2207P1 (reverse). The PCR product was assembled into the linearized pPCC1401 using HiFi DNA Assembly (New England Biolabs).

The complete nucleotide sequences of the plasmids are provided below. The plasmids were sequenced by Plasmidsaurus.

Nucleotide sequence of plasmid pPCC2000:

AAAAAAAAAAGCCCGCACTGTCAGGTGCGGGCTTTTTTCTGTGTTTGCGCAATTAACCCT
CACTAAAGGGAACAAAAGCTGGAAGCTTTCTCTGAATGGCGGGAGTATGAAAAGTTGAG
CAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCAT

AGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAA
CCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCT
GTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGC
TTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGG
CTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTT
GAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATT
AGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGC
TACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAA
GAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTG
CAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTAC
GGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATC
AAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTA
TATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCG
ATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACG
GGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATGTGTTGCCCCAGCAATCAGCGCGA
CCTTGCCCCTCCAACGTCATCTCGTTCTCCGCTCATGAGCTCAGCCAATCGACTGGCGAGC
GGCATCGCATTCTTCGCATCCCGCCTCTGGCGGATGCAGGAAGATCAACGGATCTCGGCCC
AGTTGACCCAGGGCTGTCGCCACAATGTCGCGGGAGCGGATCAACCGAGCAAAGGCATG
ACCGACTGGACCTTCCTTCTGAAGGCTCTTCTCCTTGAGCCACCTGTCCGCCAAGGCAAA
GCGCTCACAGCAGTGGTCATTCTCGAGATAATCGACGCGTACCAACTTGCCATCCTGAAGA
ATGGTGCAGTGTCTCGGCACCCCATAGGGAACCTTTGCCATCAACTCGGCAAGATGCAGC
GTCGTGTTGGCATCGTGTCCCACGCCGAGGAGAAGTACCTGCCCATCGAGTTCATGGACA
CGGGCGACCGGGCTTGCAGGCGAGTGAGGTGGCAGGGGCAATGGATCAGAGATGATCTG
CTCTGCCTGTGGCCCCGCTGCCGCAAAGGCAAATGGATGGGCGCTGCGCTTTACATTTGG
CAGGCGCCAGAATGTGTCAGAGACAACTCCAAGGTCCGGTGTAACGGGCGACGTGGCAG
GATCGAACGGCTCGTCGTCCAGACCTGACCACGAGGGCATGACGAGCGTCCCTCCCGGAC
CCAGCGCAGCACGCAGGGCCTCGATCAGTCCAAGTGGCCCATCTTCGAGGGGCCGGACG
CTACGGAAGGAGCTGTGGACCAGCAGCACACCGCCGGGGGTAACCCCAAGGTTGAGAAG
CTGACCGATGAGCTCGGCTTTTCGCCATTCGTATTGCACGACATTGCACTCCACCGCTGAT
GACATCAGTCGATCATAGCACGATCAACGGCACTGTTGCAAATAGTCGGTGGTGATAAACT
TATCATCCCCTTTTGCTGATGGAGCTGCACATGAACCCATTCAAAGGCCGGCATTTTCAGC
GTGACATCATTCTGTGGGCCGTACGCTGGTACTGCAAATACGGCATCAGTTACCGTGAGCC
GGATCAGTGAGGGTTTGCAACTGCGGGTCAAGGATCTGGATTTCGATCACGGCACGATCA
TCGTGCGGGAGGGCAAGGGCTCCAAGGATCGGGCCTTGATGTTACCCGAGAGCTTGGCAC
CCAGCCTGCGCGAGCAGGGGAATTGATCCGGTGGATGACCTTTTGAATGACCTTTAATAGA
TTATATTACTAATTAATTGGGGACCCTAGAGGTCCCCTTTTTTATTTTAAAAATTTTTTCACA
AAACGGTTTACAAGCATAAAGCTGGCCGCAAGATCCGGCCACGATGCGTCCGGCGTAGAG

GATCTGAAGACTGCAGGAAAACTAGTGGTGTCTAGAATGAGAATTCTTACGTGTGAAATT
GTTATCCGCTCACAATTCCACACATTATACGAGCCGATGATTAATTGTCAACAGCTCGCGGC
CGCGCTAGCGAATTTTCCCATGGAATGGTGCAAAACCTTTCGCGGTATGGCATGATAGCGC
CCGGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCA
GAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTT
CTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAAC
CGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGT
CTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTG
GGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGC
GGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGAC
CAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTC
TGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTG
GAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTG
TCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCC
GATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAAT
GCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGGCGCTGGG
CGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGG
ATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCACCATCAAACAGGAT
TTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCG
GTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCC
CAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAG
GTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTAAGTTAGCTCACTCA
TTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCG
GATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGGATTCACTGGCCGTCGTTT
TACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATC
CCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGT
TGC

Nucleotide sequence of plasmid pPCC2208:

AAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTT
TCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCG
TAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCC
TGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGAC
GATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCC
AGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGC
GCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAAC
AGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGG

GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTA
TGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTC
ACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGA
GCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGC
GGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATAC
AGATCCTGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAG
CGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTC
TCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCG
ATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGT
GGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATA
GTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTA
TAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAA
CGCGAATTTTAACAAAATATTAACGTTTACAGGATCTGTATGGTGCACTCTCAGTACAATCT
GCTCTGATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGCCTCGACC
TGCAGCAATTCCAACGCCATCAAAAATAATTCGCGTCTGGCCTTCCTGTAGCCAGCTTTCA
TCAACATTAAATGTGAGCGAGTAACAACCCGTCGGATTCTCCGTGGGAACAAACGGCGGA
TTGACCGTAATGGGATAGGTCACGTTGGTGTAGATGGGCGCATCGTAACCGTGCATCTGCC
AGTTTGAGGGGACGACGACAGTATCGGCCTCAGGAAGATCGCACTCCAGCCAGCTTTCCG
GCACCGCTTCTGGTGCCGGAAACCAGGCAAAGCGCCATTCGCCATTCAGGCTGCGCAACT
GTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGAT
GTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAA
CGACGGCCAGTGAATCCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCT
CACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATG
AGTGAGCTAACTTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTG
TCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGG
CGCCAGGGTGGTTTTTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGC
CTGGCCCTGAGAGAGTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAAT
CCTGTTTGATGGTGGTTAACGGCGGGATATAACATGAGCTGTCTTCGGTATCGTCGTATCCC
ACTACCGAGATATCCGCACCAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGCGCCC
AGCGCCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCTCATTCAGCATTT
GCATGGTTTGTTGAAAACCGGACATGGCACTCCAGTCGCCTTCCCGTTCCGCTATCGGCTG
AATTTGATTGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCAGACGCGCCGAGACAGA
ACTTAATGGGCCCGCTAACAGCGCGATTTGCTGGTGACCCAATGCGACCAGATGCTCCAC
GCCCAGTCGCGTACCGTCTTCATGGGAGAAAATAATACTGTTGATGGGTGTCTGGTCAGAG
ACATCAAGAAATAACGCCGGAACATTAGTGCAGGCAGCTTCCACAGCAATGGCATCCTGG
TCATCCAGCGGATAGTTAATGATCAGCCCACTGACGCGTTGCGCGAGAAGATTGTGCACCG
CCGCTTTACAGGCTTCGACGCCGCTTCGTTCTACCATCGACACCACCACGCTGGCACCCAG

TTGATCGGCGCGAGATTTAATCGCCGCGACAATTTGCGACGGCGCGTGCAGGGCCAGACT
GGAGGTGGCAACGCCAATCAGCAACGACTGTTTGCCCGCCAGTTGTTGTGCCACGCGGTT
GGGAATGTAATTCAGCTCCGCCATCGCCGCTTCCACTTTTTCCCGCGTTTTCGCAGAAACG
TGGCTGGCCTGGTTCACCACGCGGGAAACGGTCTGATAAGAGACACCGGCATACTCTGCG
ACATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGGCGCTA
TCATGCCATACCGCGAAAGGTTTTGCACCATTCCATGGGAATTCGCTAGCGCGGCCGCGAG
CTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCAC
ACAGGAGATATCATATGAAAATTAAAACTCTGGCAATCGTTGTTCTGTCGGCTCTGTCCCTC
AGTTCTACAGCGGCTCTGGCCGCTGCCACGACGGTTAATGGTGGGACCGTTCACTTTAAA
GGGGAAGTTGTTAACGCCGCTTGCGCAGTTGATGCAGGCTCTGTTGATCAAACCGTTCAG
TTAGGACAGGTTCGTACCGCATCGCTGGCACAGGAAGGAGCAACCAGTTCTGCTGTCGGT
TTTAACATTCAGCTGAATGATTGCGATACCAATGTTGCATCTAAAGCCGCTGTTGCCTTTTT
AGGTACGGCGATTGATGCGGGTCATACCAACGTTCTGGCTCTGCAGAGTTCAGCTGCGGG
TAGCGCAACAAACGTTGGTGTGCAGATCCTGGACAGAACGGGTGCTGCGCTGACGCTGG
ATGGTGCGACATTTAGTTCAGAAACAACCCTGAATAACGGAACCAATACCATTCCGTTCCA
GGCGCGTTATTTTGCAACCGGGGCCGCAACCCCGGGTGCTGCTAATGCGGATGCGACCTT
CAAGGTTCAGTATCAATAACCTACCCAGGTTCAGGGACGTCATTACGGGCAGGGATGCCC
ACCCTTGTGCGATAAAAATAACGATGAAAAGGAAGAGATTATTTCTATTAGCGTCGTTGCT
GCCAATGTTTGCTCTGGCCGGAAATAAATGGAATACCACGTTGCCCGGCGGAAATATGCAA
TTTCAGGGCGTCATTATTGCGGAAACTTGCCGGATTGAAGCCGGTGATAAACAAATGACG
GTCAATATGGGGCAAATCAGCAGTAACCGGTTTCATGCGGTTGGGGAAGATAGCGCACCG
GTGCCTTTTGTTATTCATTTACGGGAATGTAGCACGGTGGTGAGTGAACGTGTAGGTGTGG
CGTTTCACGGTGTCGCGGATGGTAAAAATCCGGATGTGCTTTCCGTGGGAGAGGGGCCAG
GGATAGCCACCAATATTGGCGTAGCGTTGTTTGATGATGAAGGAAACCTCGTACCGATTAA
TCGTCCTCCAGCAAACTGGAAACGGCTTTATTCAGGCTCTACTTCGCTACATTTCATCGCC
AAATATCGTGCTACCGGGCGTCGGGTTACTGGCGGCATCGCCAATGCCCAGGCCTGGTTCT
CTTTAACCTATCAGTAATTGTTCAGCAGATAATGTGATAACAGGAACAGGACAGTGAGTAA
TAAAAACGTCAATGTAAGGAAATCGCAGGAAATAACATTCTGCTTGCTGGCAGGTATCCTG
ATGTTCATGGCAATGATGGTTGCCGGACGCGCTGAAGCGGGAGTGGCCTTAGGTGCGACT
CGCGTAATTTATCCGGCAGGGCAAAAACAAGAGCAACTTGCCGTGACAAATAATGATGAA
AATAGTACCTATTTAATTCAATCATGGGTGGAAAATGCCGATGGTGTAAAGGATGGTCGTTT
TATCGTGACGCCTCCTCTGTTTGCGATGAAGGGAAAAAAAGAGAATACCTTACGTATTCTT
GATGCAACAAATAACCAATTGCCACAGGACCGGGAAAGTTTATTCTGGATGAACGTTAAA
GCGATTCCGTCAATGGATAAATCAAAATTGACTGAGAATACGCTACAGCTCGCAATTATCA
GCCGCATTAAACTGTACTATCGCCCGGCTAAATTAGCGTTGCCACCCGATCAGGCCGCAGA
AAAATTAAGATTTCGTCGTAGCGCGAATTCTCTGACGCTGATTAACCCGACACCCTATTACC
TGACGGTAACAGAGTTGAATGCCGGAACCCGGGTTCTTGAAAATGCATTGGTGCCTCCAA

TGGGCGAAAGCACGGTTAAATTGCCTTCTGATGCAGGAAGCAATATTACTTACCGAACAAT
AAATGATTATGGCGCACTTACCCCCAAAATGACGGGCGTAATGGAATAACGCAGGGGGAA
TTTTTCGCCTGAATAAAAAGAATTGACTGCCGGGTGATTTTAAGCCGGAGGAATAATGTCA
TATCTGAATTTAAGACTTTACCAGCGAAACACACAATGCTTGCATATTCGTAAGCATCGTTT
GGCTGGTTTTTTTGTCCGACTCGTTGTCGCCTGTGCTTTTGCCGCACAGGCACCTTTGTCA
TCTGCCGACCTCTATTTTAATCCGCGCTTTTTAGCGGATGATCCCCAGGCTGTGGCCGATTT
ATCGCGTTTTGAAAATGGGCAAGAATTACCGCCAGGGACGTATCGCGTCGATATCTATTTG
AATAATGGTTATATGGCAACGCGTGATGTCACATTTAATACGGGCGACAGTGAACAAGGGA
TTGTTCCCTGCCTGACACGCGCGCAACTCGCCAGTATGGGGCTGAATACGGCTTCTGTCGC
CGGTATGAATCTGCTGGCGGATGATGCCTGTGTGCCATTAACCACAATGGTCCAGGACGCT
ACTGCGCATCTGGATGTTGGTCAGCAGCGACTGAACCTGACGATCCCTCAGGCATTTATGA
GTAATCGCGCGCGTGGTTATATTCCTCCTGAGTTATGGGATCCCGGTATTAATGCCGGATTG
CTCAATTATAATTTCAGCGGAAATAGTGTACAGAATCGGATTGGGGGTAACAGCCATTATGC
ATATTTAAACCTACAGAGTGGGTTAAATATTGGTGCGTGGCGTTTACGCGACAATACCACCT
GGAGTTATAACAGTAGCGACAGATCATCAGGTAGCAAAAATAAATGGCAGCATATCAATAC
CTGGCTTGAGCGAGACATAATACCGTTACGTTCCCGGCTGACGCTGGGTGATGGTTATACT
CAGGGCGATATTTTCGATGGTATTAACTTTCGCGGCGCACAATTGGCCTCAGATGACAATAT
GTTACCCGATAGTCAAAGAGGATTTGCCCCGGTGATCCACGGTATTGCTCGTGGTACTGCA
CAGGTCACTATTAAACAAAATGGGTATGACATTTATAATAGTACGGTGCCACCGGGGCCTTT
TACCATCAACGATATCTATGCCGCAGGTAATAGTGGTGACTTGCAGGTAACGATCAAAGAG
GCTGACGGCAGCACGCAGATTTTTACCGTACCCTATTCGTCAGTCCCGCTTTTGCAACGTG
AAGGGCATACTCGTTATTCCATTACGGCAGGAGAATACCGTAGTGGAAATGCGCAGCAGG
AAAAAACCCGCTTTTTCCAGAGTACATTACTCCACGGCCTTCCGGCTGGCTGGACAATATA
TGGTGGAACGCAACTGGCGGATCGTTATCGTGCTTTTAATTTCGGTATCGGGAAAAACATG
GGGGCACTGGGCGCTCTGTCTGTGGATATGACGCAGGCTAATTCCACACTTCCCGATGACA
GTCAGCATGACGGACAATCGGTGCGTTTTCTCTATAACAAATCGCTCAATGAATCAGGCAC
GAATATTCAGTTAGTGGGTTACCGTTATTCGACCAGCGGATATTTTAATTTCGCTGATACAA
CATACAGTCGAATGAATGGCTACAACATCGAAACACAGGACGGAGTTATTCAGGTTAAGC
CGAAATTCACCGACTATTACAACCTCGCTTATAACAAACGCGGGAAATTACAACTCACCGT
TACTCAGCAACTCGGGCGCACATCAACACTGTATTTGAGTGGTAGCCATCAAACTTATTGG
GGAACGAGTAATGTCGATGAGCAATTCCAGGCTGGATTAAATACTGCGTTCGAAGATATCA
ACTGGACGCTCAGCTATAGCCTGACGAAAAACGCCTGGCAAAAAGGACGGGATCAGATG
TTAGCGCTTAACGTCAATATTCCTTTCAGCCACTGGCTGCGTTCTGACAGTAAATCTCAGTG
GCGACATGCCAGTGCCAGCTACAGCATGTCACACGATCTCAACGGTCGGATGACCAATCT
GGCTGGTGTATACGGTACGTTGCTGGAAGACAACAACCTCAGCTATAGCGTGCAAACCGG
CTATGCCGGGGGAGGCGATGGAAATAGCGGAAGTACAGGCTACGCCACGCTGAATTATCG
CGGTGGTTACGGCAATGCCAATATCGGTTACAGCCATAGCGATGATATTAAGCAGCTCTATT

ACGGAGTCAGCGGTGGGGTACTGGCTCATGCCAATGGCGTAACGCTGGGGCAGCCGTTAA
ACGATACGGTGGTGCTTGTTAAAGCGCCTGGCGCAAAAGATGCAAAAGTCGAAAACCAG
ACGGGGGTGCGTACCGACTGGCGTGGTTATGCCGTGCTGCCTTATGCCACTGAATATCGGG
AAAATAGAGTGGCGCTGGATACCAATACCCTGGCTGATAACGTCGATTTAGATAACGCGGT
TGCTAACGTTGTTCCCACTCGTGGGGCGATCGTGCGAGCAGAGTTTAAAGCGCGCGTTGG
GATAAAACTGCTCATGACGCTGACCCACAATAATAAGCCGCTGCCGTTTGGGGCGATGGTG
ACATCAGAGAGTAGCCAGAGTAGCGGCATTGTTGCGGATAATGGTCAGGTTTACCTCAGC
GGAATGCCTTTAGCGGGAAAAGTTCAGGTGAAATGGGGAGAAGAGGAAAATGCTCACTG
TGTCGCCAATTATCAACTGCCACCAGAGAGTCAGCAGCAGTTATTAACCCAGCTATCAGCT
GAATGTCGTTAAGGGGGCGTGATGAGAAACAAACCTTTTTATCTTCTGTGCGCTTTTTTGT
GGCTGGCGGTGAGTCACGCTTTGGCTGCGGATAGCACGATTACTATCCGCGGCTATGTCAG
GGATAACGGCTGTAGTGTGGCCGCTGAATCAACCAATTTTACTGTTGATCTGATGGAAAAC
GCGGCGAAGCAATTTAACAACATTGGCGCGACGACTCCTGTTGTTCCATTTCGTATTTTGC
TGTCACCCTGTGGTAATGCCGTTTCTGCCGTAAAGGTTGGGTTTACTGGCGTTGCAGATAG
CCACAATGCCAACCTGCTTGCACTTGAAAATACGGTGTCAGCGGCTTCGGGACTGGGAAT
ACAGCTTCTGAATGAGCAGCAAAATCAAATACCCCTTAATGCTCCATCGTCCGCGCTTTCG
TGGACGACCCTGACGCCGGGTAAACCAAATACGCTGAATTTTTACGCCCGGCTAATGGCG
ACACAGGTGCCTGTCACTGCGGGGCATATCAATGCCACGGCTACCTTCACTCTTGAATATC
AGTAACTGGAGATGCTCATGAAATGGTGCAAACGTGGGTATGTATTGGCGGCAATATTGGC
GCTCGCAAGTGCGACGATACAGGCAGCCGATGTCACCATCACGGTGAACGGTAAGGTCGT
CGCCAAACCGTGTACGGTTTCCACCACCAATGCCACGGTTGATCTCGGCGATCTTTATTCT
TTCAGTCTTATGTCTGCCGGGGCGGCATCGGCCTGGCATGATGTTGCGCTTGAGTTGACTA
ATTGTCCGGTGGGAACGTCGAGGGTCACTGCCAGCTTCAGCGGGGCAGCCGACAGTACC
GGATATTATAAAAACCAGGGGACCGCGCAAAACATCCAGTTAGAGCTACAGGATGACAGT
GGCAACACATTGAATACTGGCGCAACCAAAACAGTTCAGGTGGATGATTCCTCACAATCA
GCGCACTTCCCGTTACAGGTCAGAGCATTGACAGTAAATGGCGGAGCCACTCAGGGAACC
ATTCAGGCAGTGATTAGCATCACCTATACCTACAGCTGAACCCGAAGAGATGATTGTAATG
AAACGAGTTATTACCCTGTTTGCTGTACTGCTGATGGGCTGGTCGGTAAATGCCTGGTCATT
CGCCTGTAAAACCGCCAATGGTACCGCTATCCCTATTGGCGGTGGCAGCGCCAATGTTTAT
GTAAACCTTGCGCCCGTCGTGAATGTGGGGCAAAACCTGGTCGTGGATCTTTCGACGCAA
ATCTTTTGCCATAACGATTATCCGGAAACCATTACAGACTATGTCACACTGCAACGAGGCT
CGGCTTATGGCGGCGTGTTATCTAATTTTTCCGGGACCGTAAAATATAGTGGCAGTAGCTAT
CCATTTCCTACCACCAGCGAAACGCCGCGCGTTGTTTATAATTCGAGAACGGATAAGCCGT
GGCCGGTGGCGCTTTATTTGACGCCTGTGAGCAGTGCGGGCGGGGTGGCGATTAAAGCTG
GCTCATTAATTGCCGTGCTTATTTTGCGACAGACCAACAACTATAACAGCGATGATTTCCAG
TTTGTGTGGAATATTTACGCCAATAATGATGTGGTGGTGCCTACTGGCGGCTGCGATGTTTC
TGCTCGTGATGTCACCGTTACTCTGCCGGACTACCCTGGTTCAGTGCCAATTCCTCTTACC

GTTTATTGTGCGAAAAGCCAAAACCTGGGGTATTACCTCTCCGGCACAACCGCAGATGCG
GGCAACTCGATTTTCACCAATACCGCGTCGTTTTCACCTGCACAGGGCGTCGGCGTACAGT
TGACGCGCAACGGTACGATTATTCCAGCGAATAACACGGTATCGTTAGGAGCAGTAGGGA
CTTCGGCGGTGAGTCTGGGATTAACGGCAAATTATGCACGTACCGGAGGGCAGGTGACTG
CAGGGAATGTGCAATCGATTATTGGCGTGACTTTTGTTTATCAATAAAGAAATCACAGGAC
ATTGCTAATGCTGGTACGCAATATTACCTGAAGCTAAAAACCTGCACGTTAGCCCTTTGTAG
GCCAGATAAGACGCGTCAGCGTCGCATCTGGCATAAACAAAGCGCACTTTGCTGGTCTGT
TCCCCTCATCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAA
CAATTTCACACGTAAGAATTCTCCATCAGCTAAACGCATTTAAGGATCAAAATTATGGCAC
AAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAGAACCAGTC
TGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGCGCGAAGGAT
GACGCAGCGGGTCAGGCGATTGCTAACCGTTTCACCTCTAACATTAAAGGCCTGACTCAG
GCGGCCCGTAACGCCAACGACGGTATCTCCGTTGCGCAGACCACCGAAGGCGCGCTGTCC
GAAATCAACAACAACTTACAGCGTGTGCGTGAACTGACGGTACAGGCCACTACCGGTACT
AACTCTGAGTCTGATCTGTCTTCTATCCAGGACGAAATTAAATCCCGTCTGGATGAAATTG
ACCGCGTATCTGGTCAGACCCAGTTCAACGGCGTGAACGTGCTGGCAAAAAATGGCTCCA
TGAAAATCCAGGTTGGCGCAAATGATAACCAGACTATCACTATCGATCTGAAGCAGATTGA
TGCTAAAACTCTTGGCCTTGATGGTTTTAGCGTTAAAAATAACGATACAGTTACCACTAGT
GCTCCAGTAACTGCTTTTGGTGCTACCACCACAAACAATATTAAACTTACTGGAATTACCCT
TTCTACGGAAGCAGCCACTGATACTGGCGGAACTAACCCAGCTTCAATTGAGGGTGTTTAT
ACTGATAATGGTAATGATTACTATGCGAAAATCACCGGTGGTGATAACGATGGGAAGTATTA
CGCAGTAACAGTTGCTAATGATGGTACAGTGACAATGGCGACTGGAGCAACGGCAAATGC
AACTGTAACTGATGCAAATACTACTAAAGCTACAACTATCACTTCAGGCGGTACACCTGTT
CAGATTGATAATACTGCAGGTTCCGCAACTGCCAACCTTGGTGCTGTTAGCTTAGTAAAAC
TGCAGGATTCCAAGGGTAATGATACCGATACATATGCGCTTAAAGATACAAATGGCAATCTT
TACGCTGCGGATGTGAATGAAACTACTGGTGCTGTTTCTGTTAAAACTATTACCTATACTGA
CTCTTCCGGTGCCGCCAGTTCTCCAACCGCGGTCAAACTGGGCGGAGATGATGGCAAAAC
AGAAGTGGTCGATATTGATGGTAAAACATACGATTCTGCCGATTTAAATGGCGGTAATCTGC
AAACAGGTTTGACTGCTGGTGGTGAGGCTCTGACTGCTGTTGCAAATGGTAAAACCACGG
ATCCGCTGAAAGCGCTGGACGATGCTATCGCATCTGTAGACAAATTCCGTTCTTCCCTCGG
TGCGGTGCAAAACCGTCTGGATTCCGCGGTTACCAACCTGAACAACACCACTACCAACCT
GTCTGAAGCGCAGTCCCGTATTCAGGACGCCGACTATGCGACCGAAGTGTCCAATATGTCG
AAAGCGCAGATCATCCAGCAGGCCGGTAACTCCGTGTTGGCAAAAGCTAACCAGGTACCG
CAGCAGGTTCTGTCTCTGCTGCAGGGTTAATCGTTGTAACCTGATTAACTGAGACTGACGG
TCCGACACCCTATTACCGTAAGGAGGTCTTTTTATGCATACCTCCGAGTTGCTGAAACACAT
TTATGACATCAACTTGTCATATTTACTACTTGCACAGCGTTTGATTGTTCAGGACAAAGCGT
CCGCTATGTTTCGTCTCGGCATAAATGAAGAAATGGCGACAACGTTAGCGGCACTGACTCT

TCCGCAAATGGTTAAGCTGGCAGAAACCAATCAACTGGTTTGTCACTTCCGTTTTGACAG
CCACCAGACGATTACTCAGTTGACGCAAGATTCCCGCGTTGACGATCTCCAGCAAATTCAT
ACCGGCATCATGCTCTCAACACGCTTGCTGAATGATGTTAATCAGCCTGAAGAAGCGCTGC
GCAAGAAAAGGGCCTGATCATGAGTGAAAAAAGCATTGTTCAGGAAGCGCGGGATATTCA
GCTGGCAATGGAATTGATCACCCTGGGCGCTCGTTTGCAGATGCTGGAAAGCGAAACACA
GTTAAGTCGCGGACGCCTGATAAAACTTTATAAAGAACTGCGCGGAAGCCCACCGCCGAA
AGGCATGCTGCCATTCTCAACCGACTGGTTTATGACCTGGGAACAAAACGTTCATGCTTCG
ATGTTCTGTAATGCATGGCAGTTTTTACTGAAAACCGGTTTGTGTAATGGCGTCGATGCGG
TGATCAAAGCCTACCGTTTATACCTTGAACAGTGCCCACAAGCAGAAGAAGGACCACTGC
TGGCATTAACCCGTGCCTGGACATTGGTGCGGTTTGTTGAAAGTGGATTACTGCAACTTTC
CAGCTGCAACTGCTGCGGCGGCAATTTTATTACCCACGCTCACCAGCCTGTTGGCAGCTTT
GCCTGCAGCTTATGTCAACCGCCATCCCGGGCAGTAAAAAGACGTAAACTTTCCCAGAAT
CCTGCCGATATTATCCCACAACTGCTGGATGAACAGAGAGTACAGGCTGTTTAACTGATAC
GGTGAGGTCTAGACACCACTAGTTTTCCTGCAGTCTTCAGATCCTCTACGCCGGACGCATC
GTGGCCGGATCTTGCGGCCAGCTTTATGCTTGTAAACCGTTTTGTGAAAAAATTTTTAAAA
TAAAAAAGGGGACCTCTAGGGTCCCCAATTAATTAGTAATATAATCTATTAAAGGTCATTCA
AAAGGTCATCCACCGGATCAATTCCCCTGCTCGCAGGATCCCCGTACTATCAACAGGTTGA
ACTGCTGATCTTCAGATCCTCTACGCCGGACGCATCGTGGCCGGATCTTGCGGCCAGCTTT
ATGCTTGTAAACCGTTTTGTGAAAAAATTTTTAAAATAAAAAAGGGGACCTCTAGGGTCCC
CAATTAATTAGTAATATAATCTATTAAAGGTCATTCAAAAGGTCATCCACCGGATCAATTCCC
CTGCTCGCGCAGGCTGGGTGCCAAGCTCTCGGGTAACATCAAGGCCCGATCCTTGGAGCC
CTTGCCCTCCCGCACGATGATCGTGCCGTGATCGAAATCCAGATCCTTGACCCGCAGTTGC
AAACCCTCACTGATCCGGCTCACGGTAACTGATGCCGTATTTGCAGTACCAGCGTACGGCC
CACAGAATGATGTCACGCTGAAAATGCCGGCCTTTGAATGGGTTCATGTGCAGCTCCATCA
GCAAAAGGGGATGATAAGTTTATCACCACCGACTATTTGCAACAGTGCCGTTGATCGTGCT
ATGATCGACTGATGTCATCAGCGGTGGAGTGCAATGTCGTGCAATACGAATGGCGAAAAG
CCGAGCTCATCGGTCAGCTTCTCAACCTTGGGGTTACCCCCGGCGGTGTGCTGCTGGTCC
ACAGCTCCTTCCGTAGCGTCCGGCCCCTCGAAGATGGGCCACTTGGACTGATCGAGGCCC
TGCGTGCTGCGCTGGGTCCGGGAGGGACGCTCGTCATGCCCTCGTGGTCAGGTCTGGACG
ACGAGCCGTTCGATCCTGCCACGTCGCCCGTTACACCGGACCTTGGAGTTGTCTCTGACA
CATTCTGGCGCCTGCCAAATGTAAAGCGCAGCGCCCATCCATTTGCCTTTGCGGCAGCGGG
GCCACAGGCAGAGCAGATCATCTCTGATCCATTGCCCCTGCCACCTCACTCGCCTGCAAGC
CCGGTCGCCCGTGTCCATGAACTCGATGGGCAGGTACTTCTCCTCGGCGTGGGACACGAT
GCCAACACGACGCTGCATCTTGCCGAGTTGATGGCAAAGGTTCCCTATGGGGTGCCGAGA
CACTGCACCATTCTTCAGGATGGCAAGTTGGTACGCGTCGATTATCTCGAGAATGACCACT
GCTGTGAGCGCTTTGCCTTGGCGGACAGGTGGCTCAAGGAGAAGAGCCTTCAGAAGGAA
GGTCCAGTCGGTCATGCCTTTGCTCGGTTGATCCGCTCCCGCGACATTGTGGCGACAGCCC

TGGGTCAACTGGGCCGAGATCCGTTGATCTTCCTGCATCCGCCAGAGGCGGGATGCGAAG
AATGCGATGCCGCTCGCCAGTCGATTGGCTGAGCTCATGAGCGGAGAACGAGATGACGTT
GGAGGGGCAAGGTCGCGCTGATTGCTGGGGCAACACATCATTGCAGCACTGGGGCCAGAT
GGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAAC
GAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA
AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGT
GAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAG
CGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAAT
CTGCTGCTTGCAAAC

Primer	Sequence (5'-3')
*fimA*P1 (forward)	gttttttgaaaggaaagcagcatggattgtgtaggctggagctgc
*fimA*P2 (forward)	cgactgcccatgtcgatttagaaatagttttttgaaaggaaagcagcatgg
*fimA*P1 (reverse)	cctgggtaggttattgatactgaaccttgaaattccggggatccgtcg
*fimA*P2 (reverse)	ccgtaatgacgtccctgaacctgggtaggttattgatactgaaccttg
fliC (forward)	ggtgccgccagttctccaaccgcggtcaaactggg
fliC (reverse)	ggttggagaactggcggcaccggaagagtcagtatag
p2207P1 (forward)	aaacaaagcgcactttgctggtctgttcccctcatctgttgacaattaatcatcggctc
p2207P1 (reverse)	tcagcagttcaacctgttgatagtacggggatcctgcgagcaggggaattgatcc

Table S1 Primers used in this study.

Strains	CFU mL^{-1}
MG1655+p(blank)	2.0×10^{11}
MG1655Δ*fimA*+p(blank)	2.3×10^{11}
MG1655Δ*fimA*+p(*fim*)	5.9×10^{10}
MG1655Δ*fimA*+p(*fim-flhDC*)	4.8×10^{10}

Table S2 Number of Colony Forming Units (CFU) per mL of OD_{600} = 1 for different strains induced with 10 μM concentration of IPTG.

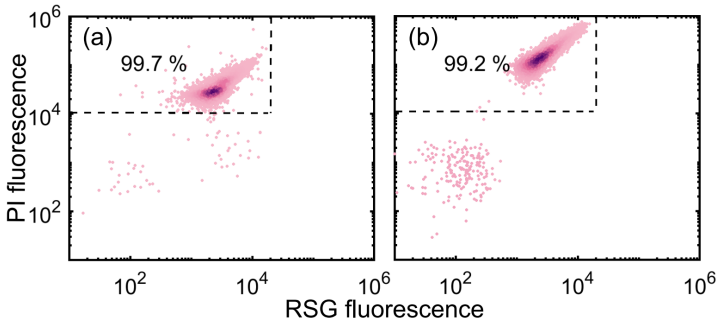

Fig. S1 (a) Cells treated with 70% ethanol for 1 h and (b) cells killed by heating at 90°C for 15 mins were stained with Propidium Iodide and Redox Sensor Green and analyzed by flow cytometry as a positive control to determine cell viability. The gating cutoffs for the PI and RSG Fluorescence were 10,000 and 20,000 respectively. The horizontal and vertical dashed lines represent the gating cutoffs for the PI and RSG Fluorescence respectively. The percentage value corresponds to the percent of dead and membrane-compromised cells in the cell population.

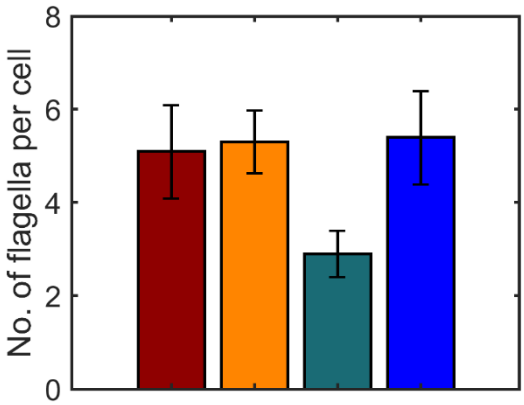

Fig. S2 Average number of flagella per cell for different strains: (■) MG1655+p(blank) + 10 µM IPTG; (■) MG1655Δ*fimA*+p(blank) + 10 µM IPTG; (■) MG1655Δ*fimA*+p(*fim*) + 10 µM IPTG; (■) MG1655Δ*fimA*+p(*fim-fliC-flhDC*) + 10 µM IPTG.

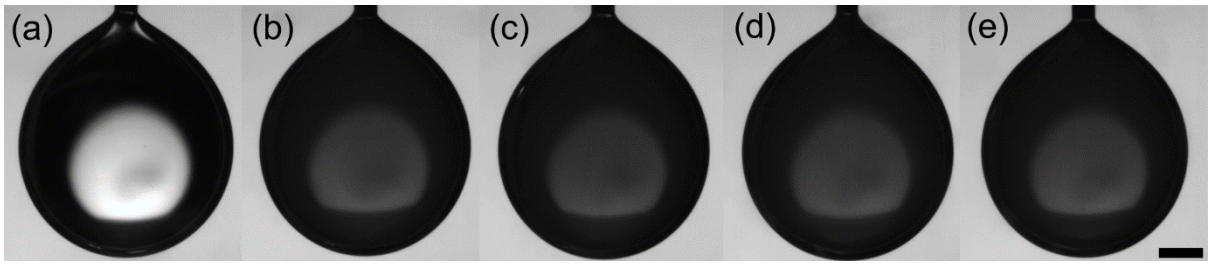

Fig. S3 Images of the pendant drop of bacterial suspension (OD_{600} = 2) of different strains (a) PBS with no cells; (b) MG1655+p(blank) + 10 µM IPTG; (c) MG1655Δ*fimA*+p(blank) + 10 µM IPTG; (d) MG1655Δ*fimA*+p(*fim*) + 10 µM IPTG; (e) MG1655Δ*fimA*+p(*fim-flhDC*) + 10 µM IPTG in hexadecane. Scale bar is 1 mm.

	MG1655+ p(blank)	MG1655Δ*fimA*+ p(blank)	MG1655Δ*fimA*+ p(*fim*)	MG1655Δ*fimA*+ p(*fim-fliC-flhDC*)
MG1655+ p(blank)	-	N	Y	Y
MG1655Δ*fimA*+ p(blank)	N	-	Y	Y
MG1655Δ*fimA*+ p(*fim*)	Y	Y	-	N
MG1655Δ*fimA*+ p(*fim-fliC-flhDC*)	Y	Y	N	-

Table S3 A 4×4 matrix indicating whether the difference in the interfacial tension values between different strains is significant at the $p < 0.05$ level.

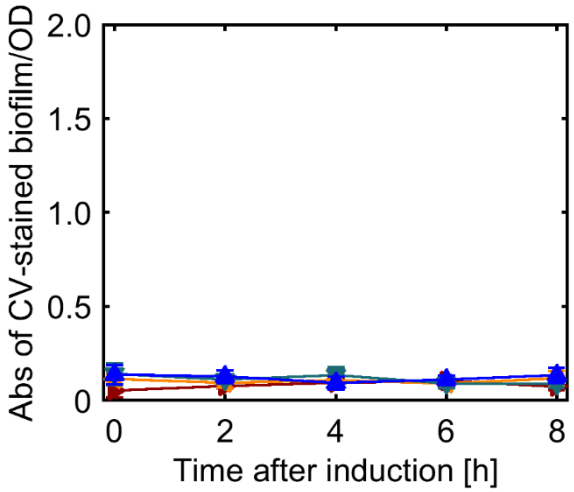

Fig. S4 MG1655Δ*fimA*+p(*fim*) and MG1655Δ*fimA*+p(*fim-fliC-flhDC*) strains do not form biofilm in the absence of IPTG. Symbols: (►) MG1655+p(blank) + 0 μM IPTG; (◄) MG1655Δ*fimA*+p(blank) + 0 μM IPTG; (▼) MG1655Δ*fimA*+p(*fim*) + 0 μM IPTG; (▲) MG1655Δ*fimA*+p(*fim-fliC-flhDC*) + 0 μM IPTG.

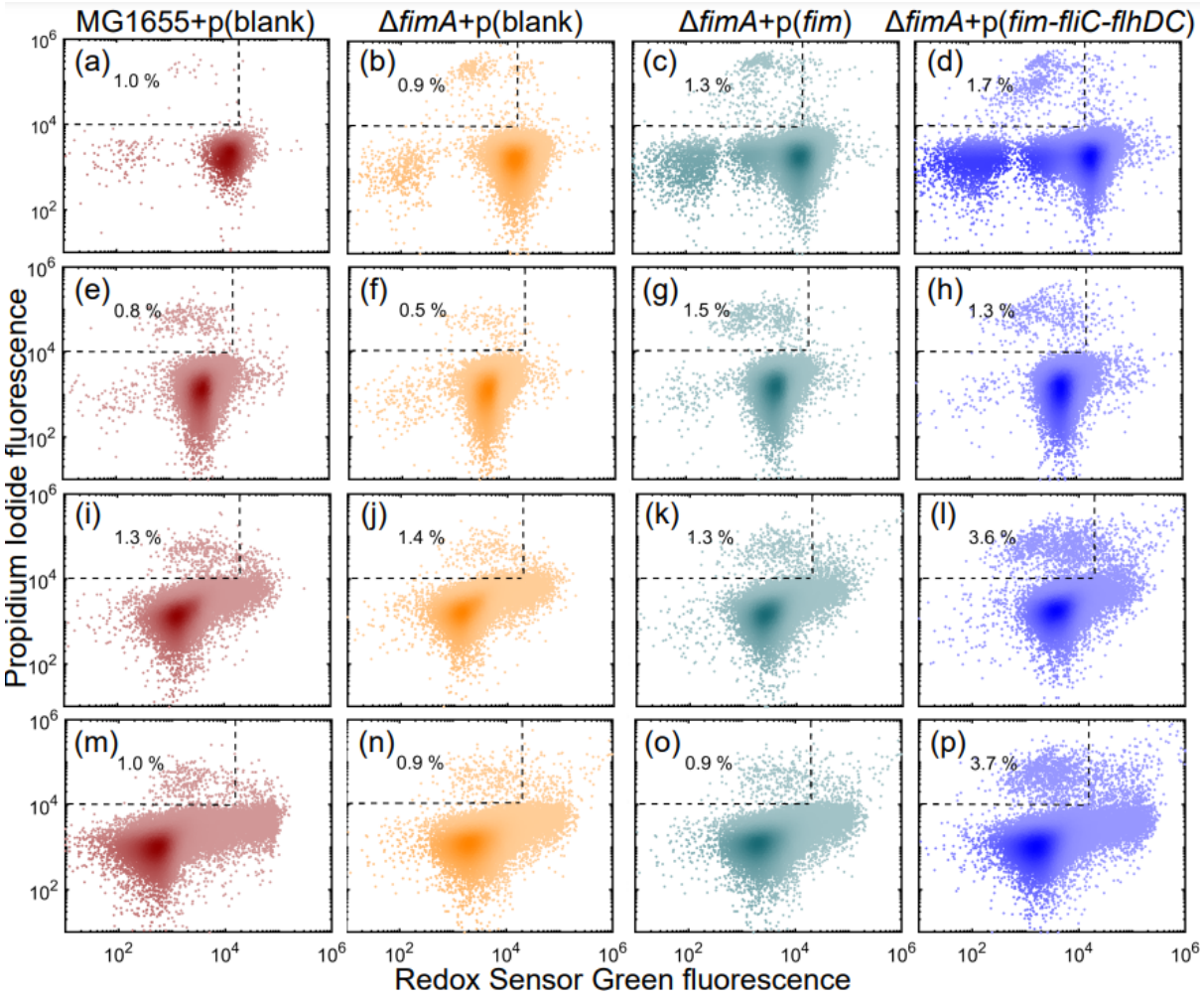

Fig. S5 PI and RSG staining of different strains (from left to right: MG1655+p(blank), MG1655Δ*fimA*+p(blank), MG1655Δ*fimA*+p(*fim*), and MG1655Δ*fimA*+p(*fim-fliC-flhDC*)) at (a – d) 0 h, (e – h) 2 h, (i – l) 6 h, and (m – p) 8 h after induction with 10 µM IPTG.

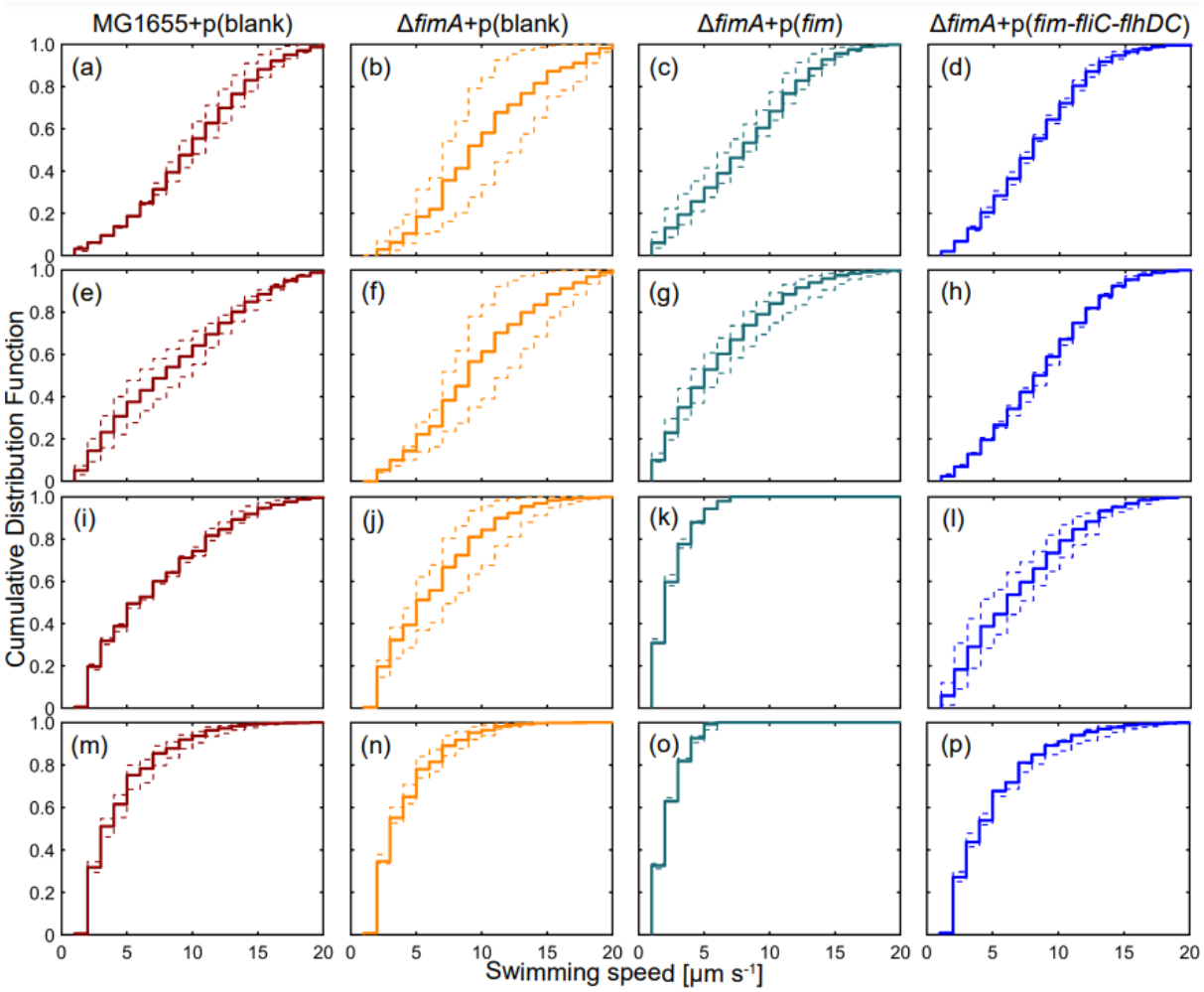

Fig. S6 The cumulative distribution function of instantaneous swimming speeds of different strains (from left to right: MG1655+p(blank), MG1655Δ*fimA*+p(blank), MG1655Δ*fimA*+p(*fim*), and MG1655Δ*fimA*+p(*fim-fliC-flhDC*)) at (a – d) 0 h, (e –h) 2 h, (i – l) 6 h, and (m – p) 8 h after induction with 10 μM IPTG.

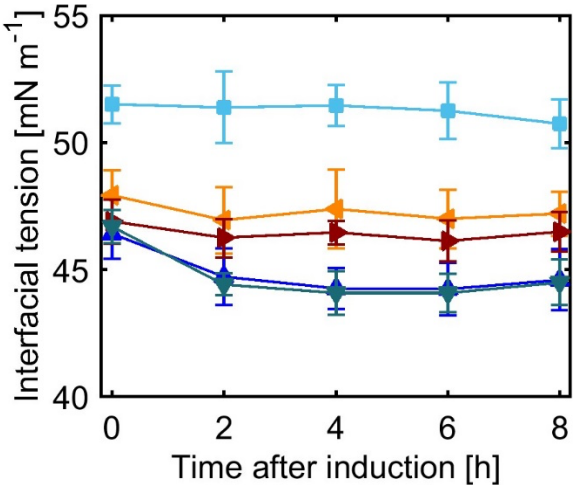

Fig. S7 Measurement of interfacial tension of the bacteria-hexadecane interface for different strains at various time points after induction with 10 μM IPTG. Symbols: (►) MG1655+p(blank) + 10 μM IPTG; (◄) MG1655Δ*fimA*+p(blank) + 10 μM IPTG; (▼) MG1655Δ*fimA*+p(*fim*) + 10 μM IPTG; (▲) MG1655Δ*fimA*+p(*fim-fliC-flhDC*) + 10 μM IPTG.

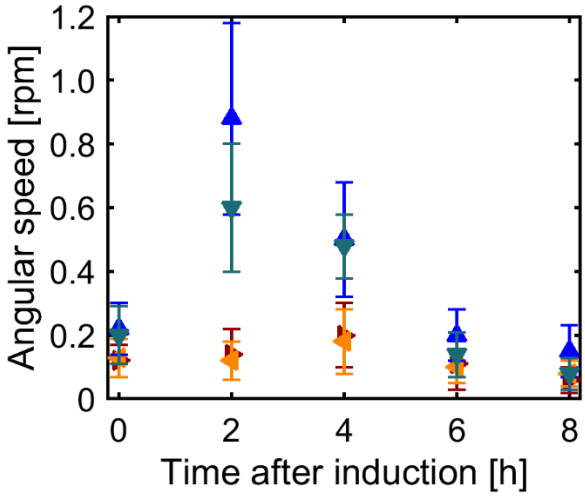

Fig. S8 Angular speed of hexadecane droplets of 25 μm diameter driven by different strains at various time points after induction with 10 μM IPTG. Symbols: (►) MG1655+p(blank) + 10 μM IPTG; (◄) MG1655Δ*fimA*+p(blank) + 10 μM IPTG; (▼) MG1655Δ*fimA*+p(*fim*) + 10 μM IPTG; (▲) MG1655Δ*fimA*+p(*fim-fliC-flhDC*) + 10 μM IPTG.

Supplementary movies:

S1.avi
S2.avi
S3.avi
S4.avi

S1.avi: Rotation behavior of hexadecane droplet driven by MG1655+p(blank) strain induced with 10 μM IPTG at cell concentration of OD_{600} = 1.

S2.avi: Rotation behavior of hexadecane droplet driven by MG1655Δ*fimA*+p(blank) strain induced with 10 μM IPTG at cell concentration of OD_{600} = 1.

S3.avi: Rotation behavior of hexadecane droplet driven by MG1655Δ*fimA*+p(*fim*) strain induced with 10 μM IPTG at cell concentration of OD_{600} = 1.

S4.avi: Rotation behavior of hexadecane droplet driven by MG1655Δ*fimA*+p(*fim-flhDC*) strain induced with 10 μM IPTG at cell concentration of OD_{600} = 1.

REFERENCES

1. K. A. Datsenko and B. L. Wanner, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 6640–6645.
2. Z. Wang, A. Doshi, R. Chowdhury, Y. Wang, C. D. Maranas and P. C. Cirino, *Protein Eng. Des. Sel.* 2020, **33**, gzaa027.