Supplementary Information for:

Design of a De Novo Aggregating Antimicrobial Peptide and a Bacterial Conjugation-Based Delivery System

Logan T. Collins, Peter B. Otoupal, Jocelyn K. Campos, Colleen M. Courtney, and Anushree Chatterjee*

Department of Chemical and Biological Engineering, University of Colorado, Boulder

*Corresponding author:

Anushree Chatterjee

3415 Colorado Avenue, 596 UCB, University of Colorado Boulder, CO 80303

Email: chatterjee@colorado.edu

Phone: (303) 735-6586

Fax: (303) 492-8425

Supplementary background on conjugative plasmids

The broad-host-range conjugative plasmid RK2 was originally isolated from antibiotic resistant *Pseudomonas aeruginosa* and *Enterobacter aerogenes* strains at the Birmingham Accident Hospital in 1969.[1] Under optimal conditions, RK2, and its shuttle plasmids have very high conjugation frequencies.[2–4] For instance, RK2 has been shown to mobilize shuttle plasmids from donor to recipient *E. coli* and from donor *E. coli* to recipient *P. aeruginosa* with conjugation frequencies of 8 and 0.2 transconjugants per donor (respectively).[4] The RK2 plasmid can be efficiently mobilized among most gram-negative and many gram-positive bacteria.[5] The pET11a backbone was chosen over RK2 itself so that its higher copy number of 15-20 copies per cell[6] (with its pBR322 OriR) relative to RK2's 4-7 copies per cell[7] and strong T7 promoter would maximize OpaL expression in targeted bacteria. This system might be particularly useful for treating infections which involve biofilms since the rate of bacterial conjugation greatly increases in biofilms, even up to 1,000-fold.[8–10] Precedent for such conjugative delivery can be found in studies that have used bacterial conjugation to deliver antibacterial CRISPR systems[11–13] and toxic hyper-replicating plasmids.[14,15]

Detailed materials and methods

Strains, plasmids, kits, and gene synthesis

The pET11a-*opaL* and pET11a-*opaLacidic* vector designs were constructed by GenScript using their artificial gene synthesis and custom cloning services. The *opaL* open reading frame, *opaLacidic* open reading frame, RK2 OriT, and chloramphenicol resistance (CmR) gene were artificially synthesized. RK2 was obtained in *E. coli* C600 (ATCC® 37125™). We used a *Mix & Go E. coli* Transformation Kit from Zymo Research to induce chemical competence in the *E. coli* C600 (RK2) before transforming with pET11a-*opaL*. To prevent loss of RK2, we grew these cells under kanamycin selection. *E. coli* C600 (RK2, pET11a-*opaL*) and *E. coli* C600 (RK2) were subsequently used as donor bacteria.

The pHL662 plasmid, carried by *E. coli* XL1 Blue, was Addgene vector 37636.[16] The *E. coli* XL1 Blue was used as recipient bacteria for measuring the mating frequency. We isolated pHL662 using a Zymo Research Zyppy Plasmid Miniprep Kit. Chemically competent *E. coli* BL21 (DE3) were acquired from NEB and transformed with pHL662. These *E. coli* BL21 (DE3) (pHL662) were used as recipients in the mating-toxicity assays. Separate samples of *E. coli* BL21 (DE3) were also transformed with pET11a-*opaL* for toxicity assays. *E. coli* NEB10-β was acquired from NEB and used as a host for propagating the pET11a-*ΔopaL* vector. The pUV145 plasmid,[17] was carried in an *E. coli* DH5α host. *E. coli* DH5α carrying pUV145 were employed in our three strain mating-toxicity assay. A list of all strains and plasmids used for this work is presented in supplementary table S1.

Culture conditions

Growth media included Luria Bertani (LB) broth (liquid medium) and LB agar (solid medium) with selective antibiotics as described for each experiment. Liquid cultures were incubated at 37°C in an orbital shaker or in a Tecan GENios plate reader. When using the plate reader, cultures were set to shake for 10 minutes, stand idle for 10 minutes, and then shake for an additional 10 seconds prior to taking a measurement. Solid cultures were grown in a stationary

incubator at 37°C. The *opaL* gene and the *opaLacidic* gene were induced using IPTG at concentrations of 1.0 or 0.1 mM as described for each experiment. GFP from pHL662 and mCherry from pUV145 were induced using IPTG at concentrations of 1.0 mM. Except where otherwise noted, ampicillin was used to maintain pET11a-*opaL* and pET11a-*opaLacidic* and kanamycin was used to maintain RK2, pHL662, and pUV145.

Molecular cloning

The pET11a-Δ*opaL* control plasmid was prepared by removing *opaL*'s open reading frame (positions 6124 to 6690). To accomplish this, pET11a-*opaL* was first propagated in *E. coli* DH5α to facilitate DNA methylation. Primers were designed to amplify the part of pET11a-*opaL* which excludes the opaL-containing sequence between pET11a-*opaL* plasmid's NdeI and BamHI cut sites. The forward primer (5'-ggaaggggatccggctgctaacaaag-3') still retained the original BamHI cut site sequence, while the reverse primer (5'-gaggagggatcctatatctccttcttaaagttaaacaaaat-3') included a small overhang which replaced the NdeI cut site with another BamHI cut site upon amplification. After amplifying this sequence, the PCR product was double digested with DpnI (a methylation-dependent restriction enzyme) and BamHI. The purpose of using DpnI was to degrade any remaining background DNA which still contained *opaL*. Next, the linear vector was ligated overnight and then electroporated into *E. coli* NEB10-β. A transformant was picked and grown in liquid media overnight. The pET11a- *ΔopaL* plasmid was miniprepped from this culture and then confirmed to have the correct size by performing gel electrophoresis alongside a sample of pET11a-*opaL*. Maps of plasmids used for this work are presented in supplementary Fig. S1.

Toxicity assay using growth curves

Overnight cultures (three biological replicates) of *E. coli* BL21 (DE3) carrying pET11a-*opaL*, *E. coli* BL21 (DE3) carrying pET11a-*opaLacidic*, *E. coli* BL21 (DE3) carrying pET11a-Δ*opaL*, and *E. coli* BL21 (DE3) without any plasmids were diluted 1×10^{-4} and incubated for 2 hours. Samples from each culture were diluted 1:50 into fresh media with 1.0 mM IPTG, 0.1 mM IPTG, and 0.0 mM IPTG in a 96 well plate. Absorbance values were measured every 20 minutes for 20 h using the Tecan Genios plate reader settings described earlier. These data were normalized by subtracting the absorbance of the media and dividing by the OD at t=0 for each sample.

Toxicity assay using Colony Forming Units (CFUs)

We tested *opaL*'s antibacterial activity in *E. coli* BL21 (DE3) carrying pET11a-*opaL* and *E. coli* BL21 (DE3) carrying pET11a-*opaLacidic*. Overnight starter cultures (three biological replicates) were diluted 1:1000 and incubated for 2 hours. Serial dilutions of these exponential cultures were plated on solid medium to obtain CFUs at t=0 h. The cultures were further diluted 1:100. Immediately after these dilutions, we split the cultures into control and experimental tubes and then added IPTG to the experimental tubes (1.0 mM final concentration) in order to induce *opaL* expression. Serial dilutions were then plated on solid media at t=2 h and t=4 h.

Computational prediction of aggregation using TANGO

The online TANGO platform (http://tango.crg.es/protected/academic/calculation.jsp) was used to predict percent aggregation for OpaL and OpaLacidic. The same parameters were used for both peptides. We assumed OpaL and OpaLacidic concentrations of 2 mM based on T7's

known expression levels.[18] Using standard physiological parameters for *E. coli*,[19,20] we entered an ionic strength of 0.25 mM, a cytosolic pH of 7.5, and a temperature of 37°C. The N- and C-termini were given the default parameter of not having any chemical modifications.

Computational structure prediction using QUARK

The online QUARK platform[21] (https://zhanglab.ccmb.med.umich.edu/QUARK/) was used to predict tertiary structures for OpaL and OpaLacidic. The raw amino acid sequences were inputted into the algorithm and the results retrieved in PDB file format. From these files, 3D graphics were created with DeepView v4.1.0.[22] DeepView was also used to compute OpaL and OpaLacidic's solvent-exposed hydrophobic surface areas.

Nile red aggregation assay

Overnight cultures (ten biological replicates) of *E. coli* BL21 (DE3) carrying pET11a-*opaL*, *E. coli* BL21 (DE3) carrying pET11a-*opaLacidic*, *E. coli* BL21 (DE3) carrying pET11a-*ΔopaL*, and *E. coli* BL21 (DE3) without any plasmids were diluted 1:10 into 900 µL of fresh media and incubated for 1 h before being pelleted, washed, and resuspended in Phosphate Buffered Saline (PBS). Nile red was added to a final concentration of 1.0 µg/mL. Absorbance was measured at 590 nm and fluorescence was measured at 590 nm excitation and 610 nm emission in a Tecan Genios plate reader. Next, IPTG was added to five of the replicates to a concentration of 1.0 mM and the other five replicates to a concentration of 0.1 mM. These samples were then incubated at 37°C for 2 h with shaking. Absorbance and fluorescence were measured again at the same wavelengths. Initial and final fluorescence values were normalized to the corresponding absorbance values and the overall normalized changes in fluorescence were calculated.

Fluorescence microscopy to visualize aggregates

Pairs of overnight cultures of *E. coli* BL21 (DE3) carrying pET11a-*opaL*, *E. coli* BL21 (DE3) carrying pET11a-*opaLacidic*, and *E. coli* BL21 (DE3) carrying pET11a-*ΔopaL* were diluted 1:10 and incubated for 90 minutes with shaking at 37˚C. IPTG was then added to half of the cultures at final concentrations of 1.0 mM and all the cultures were incubated for another 90 minutes. Next, the cells were pelleted, washed, and resuspended in PBS with 4% formaldehyde. The bacteria and intracellular aggregates were imaged using a Nikon A1R laser scanning confocal microscope with a 100x objective lens (numerical aperture 1.4), a 561 nm laser, and a 595/50 nm emission filter.

Protein gels

Overnight cultures of *E. coli* BL21 (DE3) carrying pET11a-*opaL*, *E. coli* BL21 (DE3) carrying pET11a-*opaLacidic*, and *E. coli* BL21 (DE3) carrying pET11a-*ΔopaL* were diluted 1:10 into 5 mL of fresh media and incubated for 90 minutes with shaking at 37˚C. IPTG was then added to each culture at final concentrations of 1.0 mM and the cultures were incubated for another 90 minutes. Next, the cells were pelleted, washed, and resuspended in PBS. The samples were sonicated for 30 seconds each. Total protein fractions and soluble protein fractions were run on an 8% Tris-Glycine polyacrylamide gel. The total fractions were taken directly from the lysed samples while the soluble fractions were taken from the supernatant after pelleting the total fractions. The gel was run at 4ºC for 1.5 hours using 150 V, 160 mA conditions. The gel was then fixed and stained using Coomassie Brilliant Blue R-250 dye. The gel was imaged using a

Bio-Rad Gel Doc™ EZ Gel Imager.

Mating frequency assay

Mating frequency assays were performed to confirm that RK2 and pET11a-*opaL* are capable of conjugative transfer. 1 mL overnight cultures (three biological replicates) of *E. coli* C600 donors carrying both RK2 and pET11a-*opaL*, *E. coli* C600 donors carrying the only RK2, and *E. coli* XL1 Blue recipients carrying pHL662 were pelleted and washed to remove antibiotics before being resuspended in 250 μL of media. The volumes of these cultures were adjusted to have equal OD values before mating cultures with 1:5 donor to recipient ratios were made by volume. *E. coli* C600 (RK2, pET11a-*opaL*) and *E. coli* C600 (RK2) were each separately paired with the recipients. 20 μL of the mating cultures were spotted on LB agar plates without selection and incubated for 5 hours. Next, we cut out solid agar slices with the spots and transferred them to liquid cultures without selection. After 1 h of incubation, the cultures were diluted 1:10,000 and plated on X-Gal with appropriate antibiotics.

X-Gal allowed distinction between blue donor (*E. coli* C600) colonies and white transconjugant (*E. coli* XL1 Blue) colonies. *E. coli* XL1 Blue possess the Δ*lacZ* genotype and so cannot metabolize X-Gal to produce blue pigment. Donors and transconjugants with both RK2 and pET11a-*opaL* were selected with chloramphenicol while donors and transconjugants with only RK2 were selected with ampicillin. Control X-Gal plates without antibiotics were made for each mating culture. Mating frequencies were determined by taking the ratio of transconjugant colonies to total recipient colonies.

Two strain mating-toxicity assay using CFUs

Mating-toxicity assays using CFUs demonstrated the functionality of our bacterial conjugation delivery system for transferring *opaL* to target bacteria. 1 mL overnight cultures were made with three biological replicates of *E. coli* C600 donors carrying both RK2 and pET11a-*opaL*, *E. coli* C600 donors carrying only RK2, and *E. coli* BL21 (DE3) recipients carrying pHL662. These cultures were then diluted 1:100 in 5 mL LB medium and incubated for 3 h, followed by pelleting and washing twice with LB to remove antibiotics, and then resuspension in 250 μL of fresh medium. Culture volumes were adjusted to give approximately equivalent OD values. Next, donor and recipient strains were mixed to create mating cultures with 1:3 donor to recipient ratios. Mating cultures were spotted onto 1.0 mM IPTG plates without selection and incubated for 5 h. We cut out the solid agar slices with mating spots, transferred them each into 1 mL of PBS, and vortexed thoroughly to resuspend the mated bacteria. These cells were diluted to 1×10^{-5} and plated on LB agar plates containing kanamycin (to maintain pHL662) and 1.0 mM IPTG. GFP-expressing (recipient) colonies and non-fluorescent (donor) colonies were counted using 470 nm excitation and 530 nm emission wavelengths.

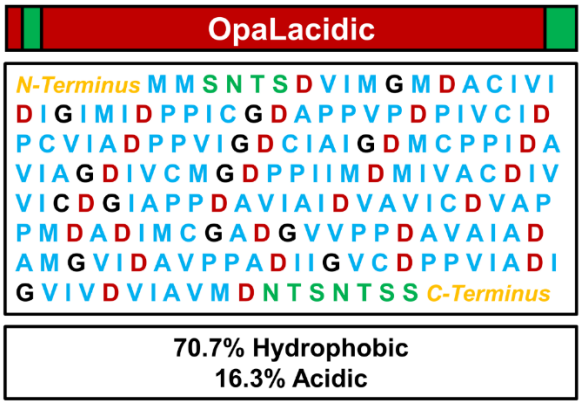
Two strain mating-toxicity assay using fluorescence growth curves

Mating-toxicity assays using fluorescence growth curves further showed the functionality of our bacterial conjugation delivery system for the *opaL* gene. 1 mL overnight cultures were made with four biological replicates of *E. coli* C600 donors carrying both RK2 and pET11a-*opaL*, *E. coli* C600 donors carrying only RK2, and *E. coli* BL21 (DE3) recipients carrying pHL662. The overnight cultures were washed twice to remove antibiotics, and resuspended in 250 μL of LB media. Culture volumes were adjusted to give approximately equivalent OD

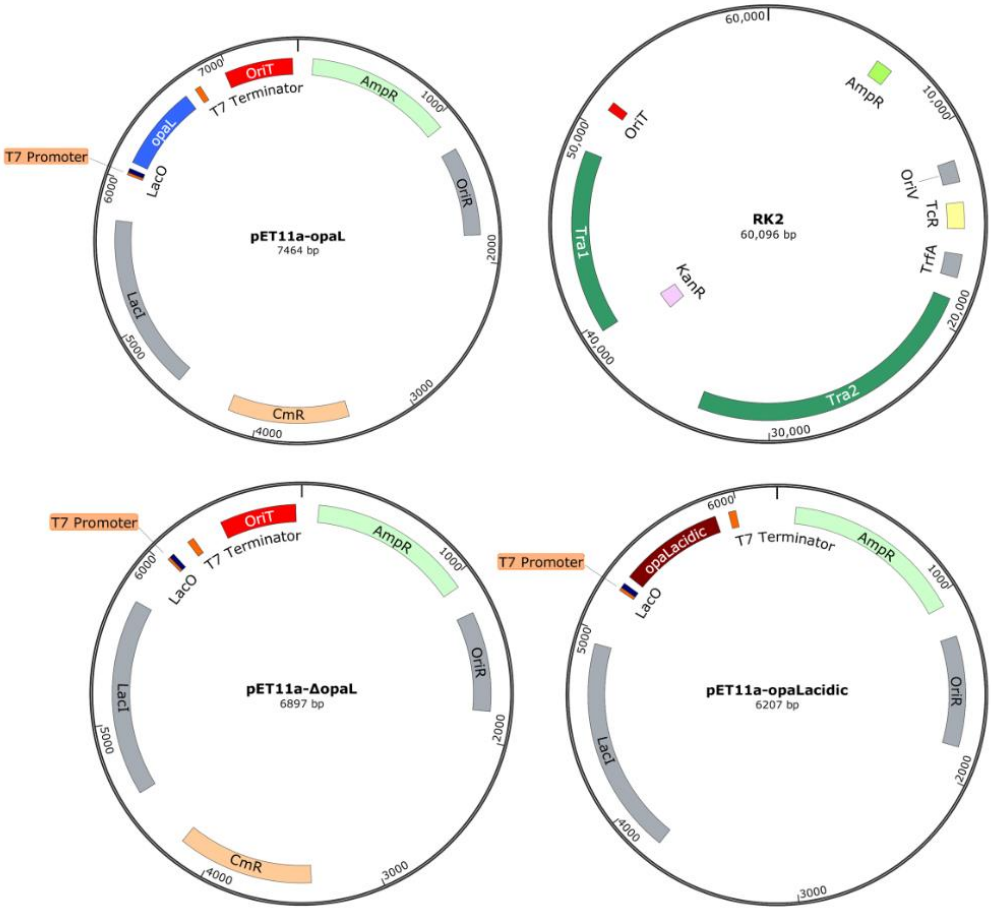
values. Next, donor and recipient strains were mixed to create mating cultures with 1:1 donor to recipient ratios. Mating cultures were spotted onto 1.0 mM IPTG plates without selection and incubated for 5 h. We cut out the solid agar slices with mating spots and transferred them to liquid media, where they were incubated for 1 h. The cultures were diluted 1:20 into fresh media with 1.0 mM IPTG in a 96 well plate. GFP fluorescence was measured with 485 nm excitation and 535 nm emission every 20 minutes for 20 h using the Tecan Genios plate reader settings described earlier. These data were normalized with the media's autofluorescence values.

Three strain mating-toxicity assay using CFUs

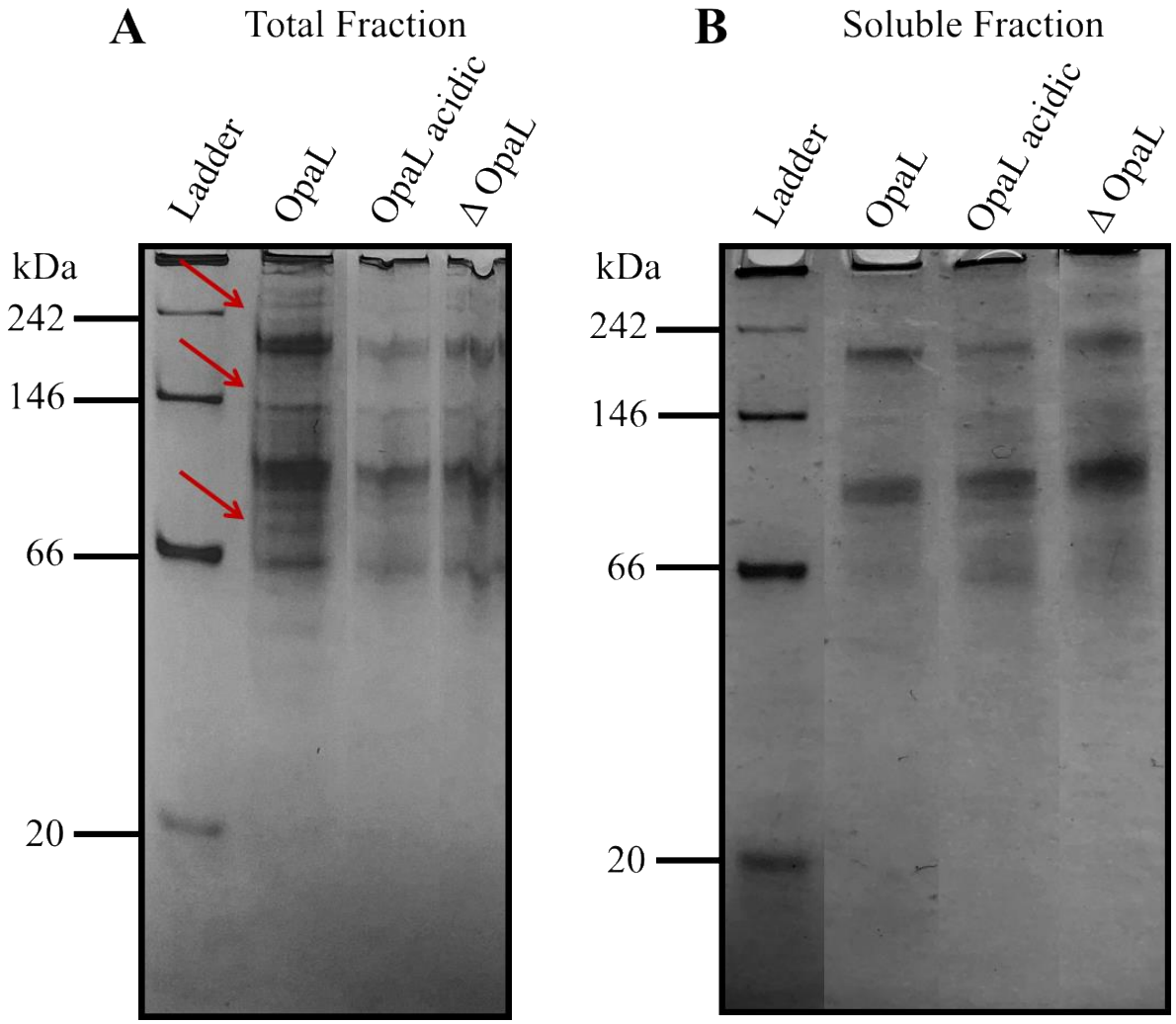
We performed a three strain mating-toxicity assay to demonstrate that this conjugation based delivery approach functions effectively when bacteria other than the donors and the targeted recipients are present. 1 mL overnight cultures were made with three biological replicates of *E. coli* C600 donors carrying both RK2 and pET11a-*opaL*, *E. coli* C600 donors carrying only RK2, *E. coli* BL21 (DE3) recipients carrying pHL662, and *E. coli* DH5α recipients carrying pUV145. As in the two strain assay, the overnight cultures were diluted 1:100 in 5 mL LB and incubated for 3 h, followed by washing twice to remove antibiotics, and resuspension in 250 μL of media. Culture volumes were adjusted to give approximately equivalent OD values. Recipient *E. coli* BL21 (DE3), recipient *E. coli* DH5α, and donor *E. coli* C600 were mixed to create mating cultures with ratios of 1:1:2 respectively. Mating cultures were spotted onto 1.0 mM IPTG plates without selection and incubated for 5 hours. Equivalent volumes of *E. coli* DH5α alone were spotted onto 1.0 mM IPTG plates without selection and incubated for 5 h. We cut out the solid agar slices with mating spots, transferred them each into 1 mL of PBS, and vortexed thoroughly to resuspend the mated bacteria. These cells were diluted to 1×10^{-5} and plated on kanamycin (to maintain pHL662 and pUV145) and 1.0 mM IPTG. GFP-expressing (target recipient) colonies and non-fluorescent (donor) colonies were counted using 470 nm excitation and 530 nm emission wavelengths, while mCherry-expressing (non-target recipient) colonies were counted using 540 nm excitation and 590 nm emission wavelengths.



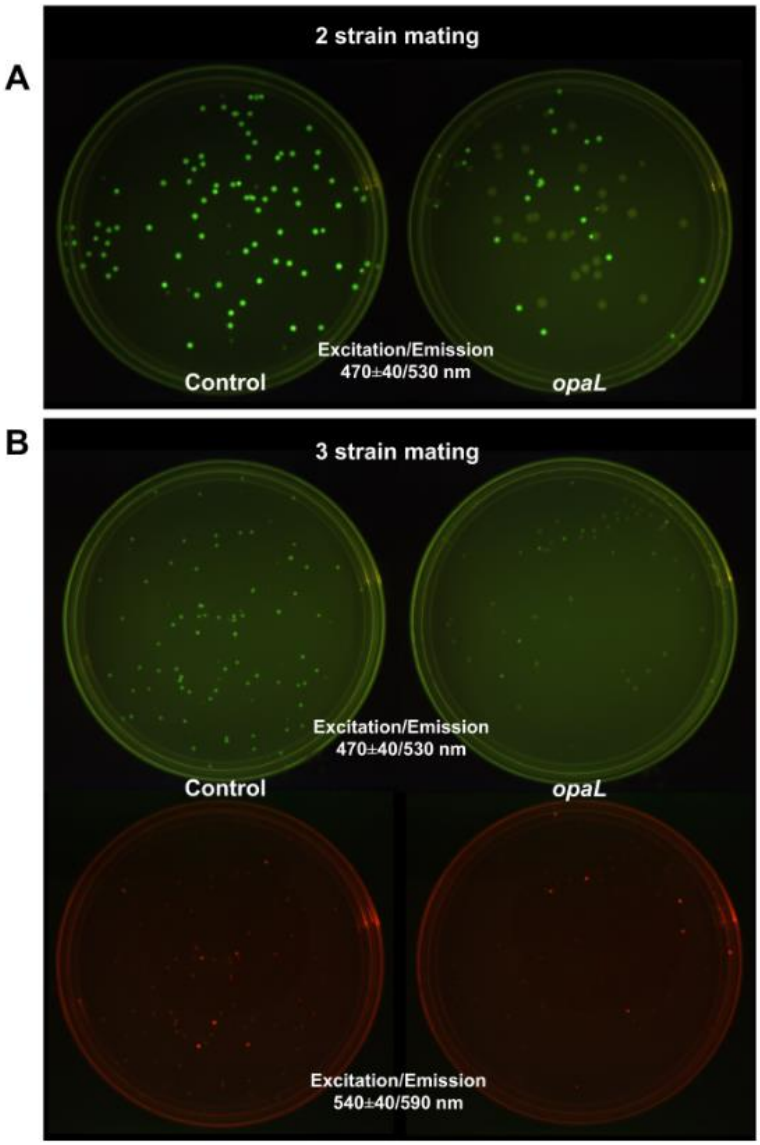
Supplementary Figure S1. The amino acid sequence of OpaLacidic. Unlike OpaL, the OpaLacidic peptide's hydrophobic patches are much more frequently interrupted by aspartic acid residues. In addition, OpaLacidic has a higher proline content, a longer stretch of C-terminal polar residues, and alternatively ordered residues within its hydrophobic patches.



Supplementary Figure S2. Plasmid maps for the broad-host range conjugative plasmid RK2, the shuttle vector pET11a-*opaL* which includes an RK2 OriT sequence and the *opaL* gene, the pET11a-*opaLacidic* plasmid, and the control vector pET11a-Δ*opaL* which does not have an open reading frame ahead of the T7 promoter.



Supplementary Figure S3. Protein extracted from OpaL, OpaLacidic, and ΔOpaL run on 8% Tris-Glycine polyacrylamide gels**. (A)** Total fraction of extracted protein. **(B)** Soluble fraction of extracted protein. Arrows in panel A indicate bands corresponding to aggregated insoluble proteins not observed in the soluble fraction of proteins shown in panel B. The number and abundance of aggregated protein bands is highest for OpaL strain (panel A). The soluble fraction of proteins is similar across OpaL, OpaLacidic and Δ OpaL. These results support that OpaL causes formation of aggregated proteins.



Supplementary Figure S4. (A) One of the replicates used to determine CFUs for the two strain mating-toxicity experiment. **(B)** One of the replicates used to determine CFUs for the three strain mating-toxicity experiment. The same pair of plates is shown under two different excitation/emission filters to distinguish between GFP expressing recipient *E. coli* BL21 (DE3) and mCherry expressing recipient *E. coli* DH5α.

Supplementary Table S1. Strains, plasmids, experiments in which each strain was utilized, and references for the sources from which these materials were obtained.

rains and Plasmids	**Experiments**	**Reference/Source**
E. coli C600, RK2	Donors for mating-toxicity assays and mating assay	ATCC® 37125™
E. coli C600, RK2, pET11a-*opaL*	Donors for mating-toxicity assays and mating assay	This study
E. coli XL1 Blue, pHL662	Recipients for mating assay	Adamson and Lim 2013
E. coli BL21 (DE3), pHL662	Recipients for mating-toxicity assays	This study
E. coli BL21 (DE3), pET11a-*opaL*	Experimental host for toxicity assay	This study
E. coli BL21 (DE3), pET11a-*opaLacidic*	Control host for toxicity assay	This study
E. coli BL21 (DE3), pET11a-***ΔopaL***	Control host for toxicity assay and nile red aggregation assay	This study
E. coli BL21 (DE3)	Control cells for toxicity assay and nile red aggregation assay	NEB#C2527I
E. coli DH5α, pUV145	Non-target recipients for three strain mating-toxicity assay	Bordoy et al. 2016

Supplementary Table S2. Percent aggregation propensities for each amino acid residue in OpaL and OpaLacidic as predicted by the TANGO algorithm.

Position	OpaL Residue	OpaL Aggregation %	OpaLacidic Residue	OpaLacidic Aggregation %
1	M	0	M	0
2	M	0	M	0
3	S	0	S	0
4	N	0.076	N	0
5	T	3.7	T	0
6	S	9.687	S	0
7	V	59.219	D	0
8	I	63.558	V	0
9	M	63.887	I	0
10	C	64.08	M	0
11	M	66.465	G	0
12	I	66.945	M	0
13	G	63.392	D	0
14	V	63.717	A	0.739
15	I	60.059	C	0.739
16	G	20.686	I	0.739
17	M	17.074	V	0.739
18	I	13.354	I	0.739
19	G	0.297	D	0
20	D	0	I	0.531
21	C	11.917	G	0.531
22	I	86.676	I	0.531
23	I	98.177	M	0.531
24	V	98.177	I	0.531
25	I	98.177	D	0
26	V	97.679	P	0
27	M	55.243	P	0
28	G	0.038	I	0
29	P	0	C	0
30	P	0	G	0
31	G	0	D	0
32	V	0	A	0
33	D	0	P	0
34	I	0.479	P	0
35	V	0.479	V	0
36	I	0.479	P	0

37	C	0.479	D	0
38	G	0.479	P	0
39	G	0.261	I	0
40	C	0.261	V	0
41	I	0.261	C	0
42	A	0.261	I	0
43	I	0.261	D	0
44	G	0	P	0
45	M	0	C	0
46	P	0	V	0
47	P	0	I	0
48	G	2.084	A	0
49	I	23.215	D	0
50	C	23.215	P	0
51	I	23.215	P	0
52	V	23.215	V	0
53	I	23.028	I	0
54	D	0	G	0
55	G	0	D	0
56	I	0	C	0
57	V	0	I	0
58	P	0	A	0
59	P	0	I	0
60	G	0	G	0
61	M	0.016	D	0
62	C	0.033	M	0
63	G	0.144	C	0
64	I	1.385	P	0
65	I	1.52	P	0
66	M	1.533	I	0
67	M	1.55	D	0
68	V	1.595	A	0
69	I	1.626	V	0
70	G	1.656	I	0
71	I	5.419	A	0
72	V	8.85	G	0
73	C	9.421	D	0
74	I	13.514	I	0
75	G	13.907	V	0
76	V	19.246	C	0
77	V	19.709	M	0
78	I	19.632	G	0
79	C	18.413	D	0

80	G	18.459	P	0
81	G	21.068	P	0
82	V	92.325	I	0
83	V	98.941	I	0
84	I	99.836	M	0
85	I	99.92	D	0
86	V	99.895	M	0
87	I	99.821	I	0
88	I	99.357	V	0
89	I	94.94	A	0
90	M	85.528	C	0
91	C	78.987	D	0
92	G	78.03	I	8.303
93	V	77.969	V	8.303
94	G	77.309	V	8.303
95	I	77.281	I	8.303
96	V	77.02	C	8.303
97	I	74.382	D	0
98	C	47.588	G	0
99	V	43.518	I	0
100	G	0.692	A	0
101	V	0.352	P	0
102	G	0.008	P	0
103	V	0.005	D	0
104	I	0.001	A	4.294
105	G	0	V	4.294
106	D	0	I	4.294
107	V	0	A	4.294
108	I	0	I	4.294
109	I	0	D	0
110	P	0	V	0.791
111	P	0.483	A	0.791
112	A	40.831	V	0.791
113	I	88.762	I	0.791
114	A	93.476	C	0.791
115	I	99.076	D	0
116	V	99.626	V	0
117	C	99.673	A	0
118	V	99.968	P	0
119	I	99.987	P	0
120	I	99.932	M	0
121	V	99.341	D	0
122	M	92.662	A	0

123	M	85.758	D	0
124	I	78.643	I	0
125	V	6.3	M	0
126	P	0.01	C	0
127	P	0	G	0
128	D	0	A	0
129	C	8.242	D	0
130	I	62.171	G	0
131	M	78.507	V	0
132	I	96.064	V	0
133	A	97.782	P	0
134	I	99.704	P	0
135	M	99.73	D	0
136	I	99.735	A	0.371
137	V	99.246	V	0.371
138	V	93.591	A	0.371
139	G	28.346	I	0.371
140	M	22.475	A	0.371
141	M	17.003	D	0
142	C	11.365	A	0
143	V	10.503	M	0
144	I	0.766	G	0
145	P	0.004	V	0
146	P	0.387	I	0
147	I	63.662	D	0
148	V	68.462	A	0
149	G	68.462	V	0
150	V	68.462	P	0
151	I	68.462	P	0
152	I	53.652	A	0
153	G	0.009	D	0
154	D	0.002	I	0
155	V	89.474	I	0
156	I	97.333	G	0
157	I	99.714	V	0
158	V	99.946	C	0
159	I	99.965	D	0
160	G	99.303	P	0
161	V	99.238	P	0
162	V	98.489	V	0
163	I	90.822	I	0
164	C	12.853	A	0
165	I	0.938	D	0

166	P	0.005	I	5.806
167	P	0	G	5.806
168	G	0	V	5.806
169	D	0	I	5.806
170	V	21.716	V	5.806
171	I	23.511	D	0
172	I	24.017	V	5.484
173	C	24.017	I	5.484
174	G	24.343	A	5.484
175	G	24.299	V	5.484
176	I	25.382	M	5.484
177	I	25.219	D	0
178	V	23.421	N	0
179	N	2.307	T	0
180	T	1.484	S	0
181	S	0.118	N	0
182	N	0	T	0
183	T	0	S	0
184	S	0	S	0
185	S	0	-	-

Supplementary References

(1) Ingram, L. C.; Richmond, M. H.; Sykes, R. B. *Antimicrob. Agents Chemother.* **1973**, *3* (2), 279–288.
(2) Waters, V. L. *Nat Genet* **2001**, *29* (4), 375–376.
(3) Guiney, D. G. *J. Mol. Biol.* **1982**, *162* (3), 699–703.
(4) Guiney, D. G.; Yakobson, E. *Proc. Natl. Acad. Sci. U. S. A.* **1983**, *80* (12), 3595–3598.
(5) Adamczyk, M.; Jagura-Burdzy, G. *Acta Biochim. Pol.* **2003**, *50* (2), 425–453.
(6) Lee, C.; Kim, J.; Shin, S. G.; Hwang, S. *J. Biotechnol.* **2006**, *123* (3), 273–280.
(7) Thomas, C. M.; Cross, M. a; Hussain, a a; Smith, C. a. *EMBO J.* **1984**, *3* (1), 57–63.
(8) Cook, L.; Chatterjee, A.; Barnes, A.; Yarwood, J.; Hu, W.-S.; Dunny, G. *Mol. Microbiol.* **2011**, *81* (6), 1499–1510.
(9) Hoiby, N.; Ciofu, O.; Bjarnsholt, T. *Future Microbiol.* **2010**, *5* (11), 1663–1674.
(10) Hausner, M.; Wuertz, S. *Appl. Environ. Microbiol.* **1999**, *65* (8), 3710–3713.
(11) Citorik, R. J.; Mimee, M.; Lu, T. K. *Nat Biotech* **2014**, *32* (11), 1141–1145.
(12) Ji, W.; Lee, D.; Wong, E.; Dadlani, P.; Dinh, D.; Huang, V.; Kearns, K.; Teng, S.; Chen, S.; Haliburton, J.; et al. *ACS Synth. Biol.* **2014**, *3* (12), 929–931.
(13) Bikard, D.; Euler, C.; Jiang, W.; Nussenzweig, P. M.; Goldberg, G. W.; Duportet, X.; Fischetti, V. A.; Marraffini, L. A. *Nat. Biotechnol.* **2014**, *32* (11), 1146–1150.
(14) Peng, Y.; Rakowski, S. A.; Filutowicz, M. *FEMS Microbiol. Lett.* **2006**, *261* (2), 245–252.
(15) Filutowicz, M.; Burgess, R.; Gamelli, R. L.; Heinemann, J. A.; Kurenbach, B.; Rakowski, S. A.; Shankar, R. *Plasmid* **2008**, *60* (1), 38–44.
(16) Adamson, D. N.; Lim, H. N. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (32), 13120–13125.
(17) Bordoy, A. E.; Varanasi, U. S.; Courtney, C. M.; Chatterjee, A. *ACS Synth. Biol.* **2016**, *5* (12), 1331–1341.
(18) Baneyx, F. *Curr. Opin. Biotechnol.* **1999**, *10* (5), 411–421.
(19) Wilks, J. C.; Slonczewski, J. L. *Journal of Bacteriology*. August 2007, pp 5601–5607.
(20) Smith, C. A. *Physiology of the Bacterial Cell. A Molecular Approach. By F C Neidhardt, J L Ingraham and M Schaechter. Pp 507. Sinauer Associates, Sunderland, MA. 1990. $43.95 ISBN 0–87893–608–4*; Headington Hill Hall, 1992; Vol. 20.
(21) Xu, D.; Zhang, Y. *Proteins Struct. Funct. Bioinforma.* **2012**, *80* (7), 1715–1735.
(22) Guex, N.; Peitsch, M. C. *Electrophoresis* **1997**, *18* (15), 2714–2723.