SUPPLEMENTAL MATERIAL

Table S1. Plasmids used in this study.

Plasmids	**Description**	**Reference**
pMQ72	*Pseudomonas* expression vector; Gm^r	(1)
pMQ30	allelic replacement; *sacB aacC1* ColE1 oriT CEN4 URA3	(1)
pKO3-*lapG*	single-crossover knockout vector for *lapG* derived from pKO3; Tet^r	(2, 3)
pKO3-*lapA*	single-crossover knockout vector for *lapA,* derived from pKO3: Tet^r	(2)
pNTerm-LapA	pMQ72 expressing NTerm-*lapA*-6xHis; Gm^r	(3)
pNTerm-LapA Δhelix	pNTerm-LapA deletion of *lapA* codons A81-95A; Gm^r	This study
pNTerm-LapA AA108-109RR	pNTerm-LapA with mutation AA108-109RR in NTerm-LapA; Gm^r	(3)
pNTerm-Lap T107A	pNTerm-LapA with mutation T107A in NTerm-LapA; Gm^r	This Study
pNTerm-Lap A108R	pNTerm-LapA with mutation A108R in NTerm-LapA; Gm^r	This Study
pNTerm-Lap A109R	pNTerm-LapA with mutation A109R in NTerm-LapA; Gm^r	This Study
pNTerm-Lap A108W	pNTerm-LapA with mutation A108W in NTerm-LapA; Gm^r	This Study
pNTerm-Lap AA108-109WW	pNTerm-LapA with mutation AA108-109WW in NTerm-LapA; Gm^r	This Study
pNTerm-Lap A109W	pNTerm-LapA with mutation A109W in NTerm-LapA; Gm^r	This Study
pNTerm-Lap A108D	pNTerm-LapA with mutation A108D in NTerm-LapA; Gm^r	This Study
pNTerm-Lap AA108-109DD	pNTerm-LapA with mutation AA108-109DD in NTerm-LapA; Gm^r	This Study
pNTerm-Lap A109D	pNTerm-LapA with mutation A109D in NTerm-LapA; Gm^r	This Study
pNTerm-Lap G110A	pNTerm-LapA with mutation G110A in NTerm-LapA; Gm^r	This Study
pMQ30-LapA AA108-109RR	allelic replacement construct for introducing lapA AA108-109RR; Gm^r	(3)

pMQ30-LapA AA108-109DD	allelic replacement construct for introducing AA108-109DD; Gm^r	This Study
pMQ30-LapA A109D	allelic replacement construct for introducing A109D; Gm^r	This Study
pMQ30-LapA A108W	allelic replacement construct for introducing A108W; Gm^r	This Study
pMQ30-LapA Repeats	allelic replacement construct for deleting repeats; Gm^r	This Study
pMQ30-LapA vWA	allelic replacement construct for deleting vWA; Gm^r	This Study
pMQ30-LapA CTerm	allelic replacement construct for deleting CTerm; Gm^r	This Study
pMQ30-LapA RTX	allelic replacement construct for deleting RTX; Gm^r	This Study
pMQ30-LapA T1SS	allelic replacement construct for deleting T1SS; Gm^r	This Study
pMQ30-LapA A108-120A	allelic replacement construct for deleting residues A108-120A; Gm^r	This Study

Table S2. Oligonucleotide primers used in this study.

	Primer	Sequence
1	pMQ30 verify Fwd	GAGTCAGTGAGCGAGGAAG
2	pMQ72 and pMQ30 verify Rev	CAGACCGCTTCTGCGTTCTG
3	pMQ72 verify Fwd	GCAACTCTCTACTGTTTCTCC
4	Delete Repeats Upstream Fwd	AGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGG ATGGCACCCAGAAGGTCAATAGTTT
5	Delete Repeats Upstream Rev	TCAGGAACATCGTATGGGTAGAGCAGGATGGTGTCGGTG ACCTGAGTCG
6	Delete Repeats Downstream Fwd	CGACTCAGGTCACCGACACCATCCTGCTCTACCCATACG ATGTTCCTGA
7	Delete Repeats Downstream Rev	AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCA GCCGTCCAGATACTGCAGTTTGC
8	Delete Repeats verify Fwd	GGATGGCACCCAGAAGGTCAATAGTTT
9	Delete Repeats verify Fwd	CTGCTCTACCCATACGATGTTCCTGA
10	Delete Repeats verify Fwd	TGATGCTCGATGCGACCGCCG
11	Delete Repeats verify Fwd	CGTGACCGATGGCGGCAACTACT
12	Delete Repeats, delete helix verify Rev	GATGGTGTCGGTGACCTGAGTCG
13	Delete Repeats verify Rev	AGCCGTCCAGATACTGCAGTTTGC
14	Delete Repeats verify Rev	CGGTGCCGTTGGTGCTGTTGG
15	Delete Repeats, delete CTerm verify Rev	CTGAGCAGCGTAATCTGGAACGTCATA
16	Delete Repeats verify Rev	TGGCATCCGGCACGAAGCGCA
17	Delete Repeats verify Rev	CATCGAGCATCACGAAGCTG
18	Delete vWA Upstream Fwd	AGCTATGACCATGATTACGAATTCGAGCTCGGTACCCTG GCACCATCAAGATCGACATCACG

19	Delete vWA Upstream Rev	CTTCGACGTTCGACAGACCGTTGGTGCCCGGCACCACG
20	Delete vWA Downstream Fwd	CTGACCGTGGTGCCGGGCACCAACGGTCTGTCGAACGT CGAAGCGATC
21	Delete vWA Downstream Rev	AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCC GTTGCACGTAAAACATTGAGCATCCCT
22	Delete vWA verify Fwd	TGGCACCATCAAGATCGACATCACG
23	Delete vWA verify Fwd	GGTCTGTCGAACGTCGAAGCGATC
24	Delete vWA verify Fwd	ACTTTCAGCAAGGCCGACATCGAC
25	Delete vWA verify Fwd	ACAACCAGAGCGGCCCGGG
26	Delete vWA verify Fwd	GTCGACGTCAGCAAGGTCACCAC
27	Delete vWA verify Fwd	ATTCATAACCTGATTGCCGGCAGCGAT
28	Delete vWA verify Rev	GTTGGGGCACCACG
29	Delete vWA verify Rev	CGTTGCACGTAAAACATTGAGCATCCCT
30	Delete vWA verify Rev	TGATGCTCAGGGTTTCCGAGCCGT
31	Delete vWA verify Rev	CTTGCCGCCGATGGTCACCAG
32	Delete vWA verify Rev	TGCAACAGGTCGCGCAGGTCGAT
33	Delete RTX Upstream Fwd	AGCTATGACCATGATTACGAATTCGAGCTCGGTACCCTAC AACGGCCAGTTCAACCTGACCG
34	Delete RTX Upstream Rev	AGCTATGACCATGATTACGAATTCGAGCTCGGTACCCCG TGAAGCTGACCGCAACCACG
35	Delete RTX Downstream Fwd	TGGCCTTGAAGTCCTTGATCACGTCGCACTGAGCAGCGT AATCTGGAAC
36	Delete RTX Downstream Rev	AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCA GCATCGATCTGCCGTTGATTGCC
37	Delete RTX verify Fwd	TACAACGGCCAGTTCAACCTGACCG
38	Delete RTX verify Fwd	ACCTTCGTCTGGAAGGCGGGC
39	Delete RTX verify Fwd	CGGCGGCACCAACTACGAGG
40	Delete RTX verify Fwd	GCAGGCCACAGCTTCACCGTGA

41	Delete RTX, delete CTerm verify Rev	AGCATCGATCTGCCGTTGATTGCC
42	Delete RTX, delete CTerm verify Rev	GCGTTGCACGTAAAACATTGAGCATCC
43	Delete RTX, delete CTerm verify Rev	CTGGCGTACCTGAAGATCGATGGC
44	Delete T1SS Upstream Fwd	AGCTATGACCATGATTACGAATTCGAGCTCGGTACCC CTCGGGGACGGTGAACATCTTCCT
45	Delete T1SS Upstream Rev	GGGCGGTCGTTGTTCACGCATCAGTTGCCGGTGTCGCC CGC
46	Delete T1SS Downstream Fwd	GCGGGCGACACCGGCAACTGATGCGTGAACAACGACCG CCC
47	Delete T1SS Downstream Rev	AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCA GCAGATGGCCGGACATGAAGAATCTT
48	Delete T1SS verify Fwd	TGCTGGACTCGATGGCGTCCG
49	Delete T1SS verify Fwd	AAGGACTCGCTGACGTCGGTGTTC
50	Delete T1SS verify Fwd	TCGAGCCTGGCGGGCAGC
51	Delete T1SS verify Fwd	CTCGGGGACGGTGAACATCTTCCT
52	Delete T1SS verify Fwd	GGCGGCCCAGGCCAAGCT
53	Delete T1SS verify Fwd	GCCAGGTTGCCGATCATGCTGAA
54	Delete T1SS verify Rev	AGCAGATGGCCGGACATGAAGAATCTT
55	Delete T1SS verify Rev	GCAGGGTCAGACCATTCC
56	Delete T1SS verify Rev	GGATCGATGTTGGTCTGTGGCGTC
57	Delete T1SS verify Rev	TGTAGGTGGCGAAGCTGCTGCC
58	Delete T1SS verify Rev	GGGGCAGCTGTTCGGTGAGC
59	Delete A108-120A Upstream Fwd	AGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGG CAACCGGAATGACCGTATGTCG
60	Delete A108-120A Upstream	ACGAAGCTGTGGCCGCCACCGGTGGATTCAAGGGCGGT

	Rev	AGTCG
61	Delete A108-120A Downstream Fwd	CGACTACCGCCCTTGAATCCACCGGTGGCGGCCACAGC TTCGT
62	Delete A108-120A Downstream Rev	CTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGATG GTGTCGGTGACCTGAGTCGT
63	*lapA* AA108-109DD point mutant Upstream Fwd	CAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCG CTGGATCTGGTCGTAGATGCGC
64	*lapA* AA108-109DD point mutant Upstream Rev	GTCGTCGGTGGATTCAAGGGCGGTAGTC
65	*lapA* AA108-109DD point mutant Downstream Fwd	GACTACCGCCCTTGAATCCACCGACGACGGCCCGAGCG CCGCT
66	*lapA* AA108-109DD point mutant Downstream Rev	AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCG TCGCTGGCGTCGTGTTCG
67	*lapA* A109D point mutant Upstream Fwd	CAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCG CTGGATCTGGTCGTAGATGCGC
68	*lapA* A109D point mutant Upstream Rev	GTCTGCGGTGGATTCAAGGGCGGTAGTC
69	*lapA* A109D point mutant Downstream Fwd	GACTACCGCCCTTGAATCCACCGCAGACGGCCCGAGCG CCGCT
70	*lapA* A109D point mutant Downstream Rev	AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCG TCGCTGGCGTCGTGTTCG
71	*lapA* A108W point mutant Upstream Fwd	CAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCG CTGGATCTGGTCGTAGATGCGC
72	*lapA* A108W point mutant Upstream Rev	GGCCCAGGTGGATTCAAGGGCGGTAGTC
73	*lapA* A108W point mutant Downstream Fwd	GACTACCGCCCTTGAATCCACCTGGGCCGGCCCGAGCG CCGCT
74	*lapA* A108W point mutant	AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCG

	Downstream Rev	TCGCTGGCGTCGTGTTCG
75	*lapA* AA108-109DD, *lapA* A109D, *lapA* A108W verify Rev	CGGTGCGTTGCCAGCCAATG
76	Delete A108-120A verify Rev	GTCTGGCCTTCGGTTACCGAGGT
77	Delete Repeats verify Rev	ACCGCTGGAGTTGTTGGCGTCG
78	Delete Repeats verify Rev	GGCCTTGCTGAAAGTCTGGTTGTTGG
79	Delete vWA, delete T1SS verify Rev	CCCGGATCATTTCCAGGTCGCTCT
80	Delete vWA, delete T1SS verify Rev	GTTGCCGGTGTCGCCCGC
81	*lapA* AA108-109DD, *lapA* A109D, *lapA* A108W verify Fwd	ACTCGGCCGAACGCAGGATC
82	Delete Repeats, Delete A108-120A, delete helix verify Fwd	CGAAGGGTAGAGCCTTGAACAGGT
83	Delete A108-120A, delete helix verify Rev	TGTCGATGGTGTCGGTGACCTGAGT
84	Delete Repeats, Delete A108-120A, *lapA* AA108-109DD, *lapA* A109D, *lapA* A108W verify Fwd	CATCAGGTCTATGGAAGTCAACTGAAC
85	Delete Repeats, Delete A108-120A, *lapA* AA108-109DD, *lapA* A109D, *lapA* A108W verify Fwd	AGCGCCACGCCGAGCATCA
86	Delete A108-120A verify Fwd	GCCGGTGTCGACCCGACTAC
87	Delete Repeats verify Fwd	GGCCCGAGCGCCGCTG
88	*lapA* AA108-109DD, *lapA* A109D, *lapA* A108W verify Rev	AATCACGATCACCGCGCCGTT
89	pNTerm-Lap A108W Upstream Fwd	ATACCCGTTTTTTTGGGCTAGCGAATTCGAGCTCGGTACCC CGGAGAGTCTTCAATGAGCAGTG

90	pNTerm-Lap A108W Upstream Rev	GGCCCAGGTGGATTCAAGGGCGGTAGTC
91	pNTerm-Lap A108W Downstream Fwd	GACTACCGCCCTTGAATCCACCTGGGCCGGCCCGAGCG CCGCT
92	pNTerm-Lap A108W Downstream Rev	CTCTAGAGGATCCCCTTAATGATGATGATGATGATGTCCA ACAGTGTCGTTCGGTGCCAG
93	pNTerm-Lap AA108-109DD Upstream Fwd	ATACCCGTTTTTTTGGGCTAGCGAATTCGAGCTCGGTACC C CGGAGAGTCTTCAATGAGCAGTG
94	pNTerm-Lap AA108-109DD Upstream Rev	GTCGTCGGTGGATTCAAGGGCGGTAGTC
95	pNTerm-Lap AA108-109DD Downstream Fwd	GACTACCGCCCTTGAATCCACCGACGACGGCCCGAGCG CCGCT
96	pNTerm-Lap AA108-109DD Downstream Rev	CTCTAGAGGATCCCCTTAATGATGATGATGATGATGTCCA AC GTGTCGTTCGGTGCCAG
97	pNTerm-Lap A109D Upstream Fwd	ATACCCGTTTTTTTGGGCTAGCGAATTCGAGCTCGGTACC CCGGAGAGTCTTCAATGAGCAGTG
98	pNTerm-Lap A109D Upstream Rev	GTCTGCGGTGGATTCAAGGGCGGTAGTC
99	pNTerm-Lap A109D Downstream Fwd	GACTACCGCCCTTGAATCCACCGCAGACGGCCCGAGCG CCGCT
100	pNTerm-Lap A109D Downstream Rev	CTCTAGAGGATCCCCTTAATGATGATGATGATGATGTCCA ACAGTGTCGTTCGGTGCCAG
101	pNTerm-Lap G110A Upstream Fwd	ATACCCGTTTTTTTGGGCTAGCGAATTCGAGCTCGGTACC C CGGAGAGTCTTCAATGAGCAGTG
102	pNTerm-Lap G110A Upstream Rev	GGCGGCTGCGGTGGATTCAAGGGC
103	pNTerm-Lap G110A Downstream Fwd	GACTACCGCCCTTGAATCCACCGCAGCCGCCCCGAGCG CCGCT

104	pNTerm-Lap G110A Downstream Rev	CTCTAGAGGATCCCCTTAATGATGATGATGATGATGTCCAACAGTGTCGTTCGGTGCCAG
105	Delete helix A81-95A Upstream Fwd	AGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGCAACCGGAATGACCGTATGTCG
106	Delete helix A81-95A Upstream Rev	GTAGTCGGGTCGACACCGGCGGTGGCTTCGGCCAGGTCAGT
107	Delete helix A81-95A Downstream Fwd	GCACTGACCTGGCCGAAGCCACCGCCGGTGTCGACCCGACTAC
108	Delete helix A81-95A Downstream Rev	CTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGATGGTGTCGGTGACCTGAGTCGT
109	Delete helix verify Fwd	GGCAACCGGAATGACCGTATGTCG
110	Delete helix verify Rev	GGTGGCTTCGGCCAGGTCAGT
111	Delete helix verify Fwd	GCCGGTGTCGACCCGACTAC
112	Delete helix verify Rev	GATGGTGTCGGTGACCTGAGTCGT
113	Delete helix verify Rev	TGCCCGCCGGTGATGTTTTGC
114	Delete CTerm Upstream Fwd	AGCTATGACCATGATTACGAATTCGAGCTCGGTACCCCGTGAAGCTGACCGCAACCACG
115	Delete CTerm Upstream Rev	TGGCCTTGAAGTCCTTGATCACGTCGCACTGAGCAGCGTAATCTGGAAC
116	Delete CTerm Downstream Fwd	GTTCCAGATTACGCTGCTCAGTGCGACGTGATCAAGGACTTCAAGGCCA
117	Delete CTerm Downstream Rev	AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCAGCATCGATCTGCCGTTGATTGCC
118	Delete CTerm verify Fwd	AAGACCACCGGCACCGTGACC
119	Delete CTerm verify Fwd	ACACCACCACTGTCAGCATCACCGG
120	Delete CTerm verify Fwd	GGTGCAGTCATCACTATCGAAGCC
121	Delete CTerm verify Fwd	CGTGAAGCTGACCGCAACCACG

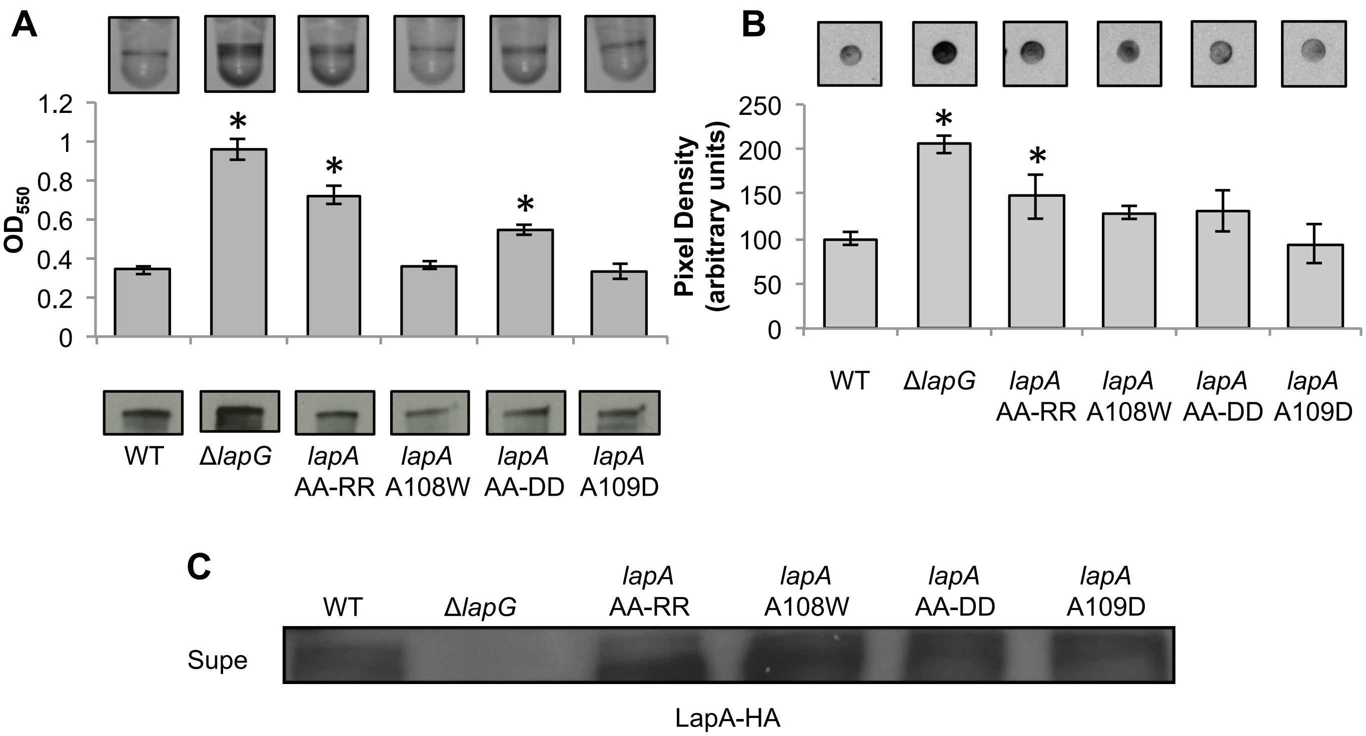
Table S3. Summary of mutations in the N-terminus of LapA and their phenotype.

NTerm-LapA Mutants	Processed by LapG in vitro?
Δhelix A81-95A	No
T107A	Yes
A108R	No
AA108-109RR	No
A109R	No
A108W	No
AA108-109WW	Yes[a]
A109W	Yes
A108D	Yes[b]
AA108-109DD	No
A109D	Yes[b]
G110A	Yes[b]

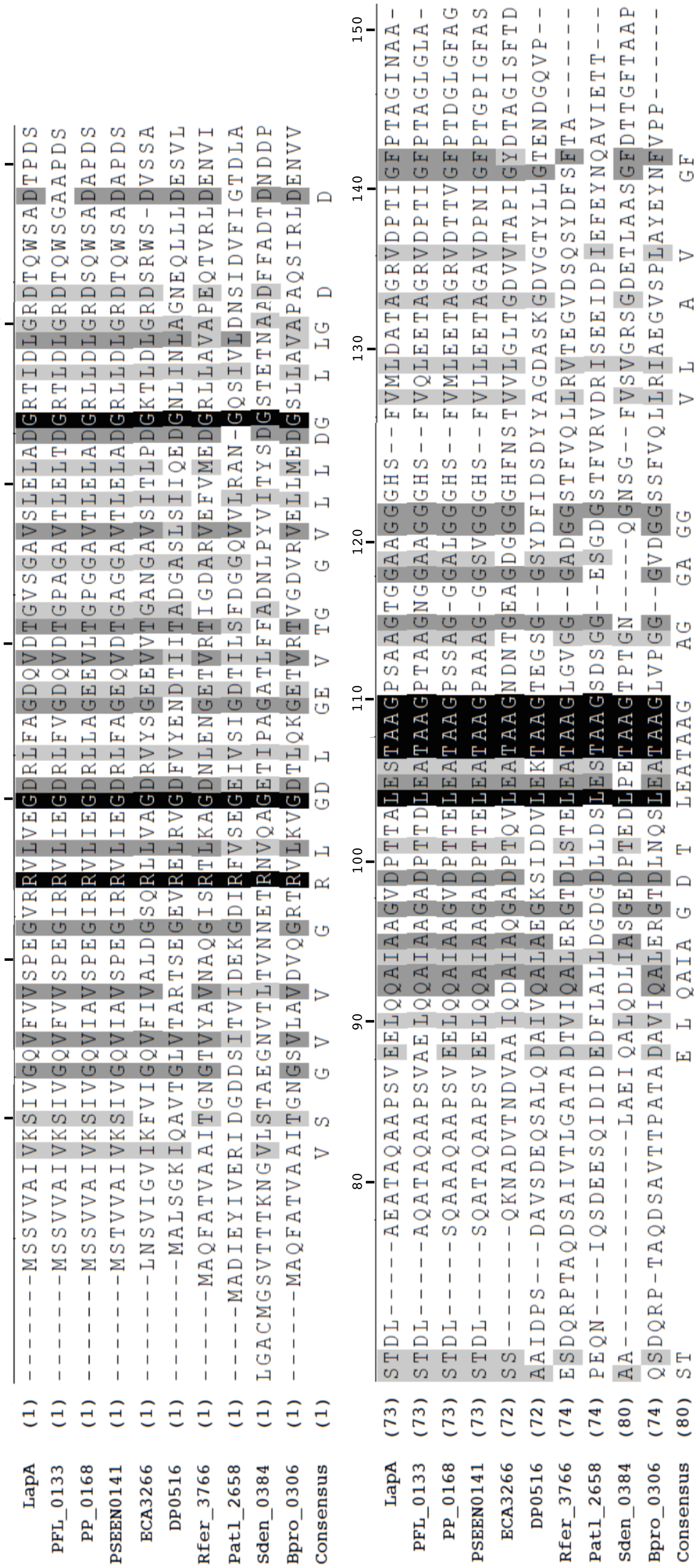
[a]This mutation likely de-stabilized the protein as the levels of NTerm-LapA were very low and the protein difficult to detect, although it appeared that the protein was still cleaved.

[b]Very faint high (~25 kDa) and low (~15 kDa) molecular weight bands in WT background; only high molecular weight band present in Δ*lapG* background. These data indicated that the protein was unstable but still at least partially cleaved.

Supplemental Figure 1. Mutational analysis of the LapG cleavage site. A. Shown are images of biofilm formation of WT *P. fluorescens* Pf0-1, Δ *lapG*, and LapA missense mutant variants in K10T-1 medium, with images of representative wells (top) and biofilm quantification (middle). The X axis shows the *P. fluorescens* strains and the Y axis shows the optical density at 550 nm (OD 550) of the solubilized crystal violet used to determine the bacterial biofilm biomass. The A108W and A109D variants of LapA were not significant different from the WT strain, and while the AA108-109DD double mutant did significantly enhance biofilm formation relative to the WT, the level of biofilm formation was less than that observed for the AA-RR LapA mutant or the strain in which the *lapG* gene was deleted. Western blot assay of the levels of LapA in each strain in the whole cell fraction is shown at the bottom of the panel. LapA was detected via an engineered HA tag. The expression of the LapA missense mutations in cellular extracts was similar to WT levels. * indicates significantly different from the WT ($P < 0.05$). **B.** Representative dot blot assays (top) and quantification of the pixel density of four replicates (bottom) to assess the level of cell surface LapA of WT *P. fluorescens* Pf0-1, Δ*lapG*, and LapA missense variants. The change in LapA present at the cell surface of the missense variants was not significantly different from the WT. LapA was detected via an engineered HA tag. * indicates significantly different from the WT ($P < 0.05$). **C.** Western blot assay to detect a HA-tagged LapA in the supernatant fraction of WT *P. fluorescens* Pf0-1, Δ*lapG*, and LapA missense variants. Missense mutations in LapA did not alter LapG-dependent cleavage of LapA due to the presence of LapA in the supernatant fraction.



Supplemental Figure 2. Extended LapA alignment. Shown is an extended alignment of the first ~150 amino acids of the N-terminal domain of LapA protein of *P. fluorescens* Pf0-1 and homologs from a variety of organisms, including pathogens. The consensus sequence is shown in the bottom row. Numbers above the alignment corresponds to the Pf0-1 LapA amino acids from. Legend: LapA, *P. fluorescens* Pf0-1; PFL_0133, *P. fluorescens* Pf-5; PP_0168, *P. putida* KT2440; PSEEN0141, *Pseudomonas entomophila* L-48; ECA3266, *Pectobacterium atrosepticum* SCRI1043; DP0516, *Desulfotalea psychrophila* LSv54; Rfer_3766, *Rhodoferax ferrireducens* DSM 15236; Patl_2528, *Pseudoalteromonas atlantica* T6c; Sden_0384, *Shewanella denitrificans* OS217; Bpro_0306, *Polaromonas* sp. JS666.



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