

separated into a list and the R programming language package VennDiagram was used to find the overlap between the groups. b) Counts of LapG-like proteins in various microbial genera. Proteins belonging to the genus *Desulfovibrio* are highlighted in blue, ABC transporters in green, and adhesins in yellow. c) Genomic arrangement of Lap components of *P. fluorescens* Pf0-1, *D. vulgaris* Hildenborough, *D. sulfuricans* ND132 and *Desulfomicrobium* sp. ZS1. LapG-like proteins are highlighted in blue. d) Secondary structure prediction using JPred4 of DvhA aa 1-121 and LapA aa1 -123. Green arrows represent beta sheets, red arrows represent alpha-helix. Double alanine motifs are highlighted in blue. e) AlphaFold structure prediction of the first 150 amino acids of LapA and DvhA displaying a folded globular domain at the N-terminus of the two proteins (shown in orange), likely serving as a retention module. f) Comparison of domain architectures of LapD (top) and DvhD (bottom) and using Simple Modular Architecture Research Tool (SMART).

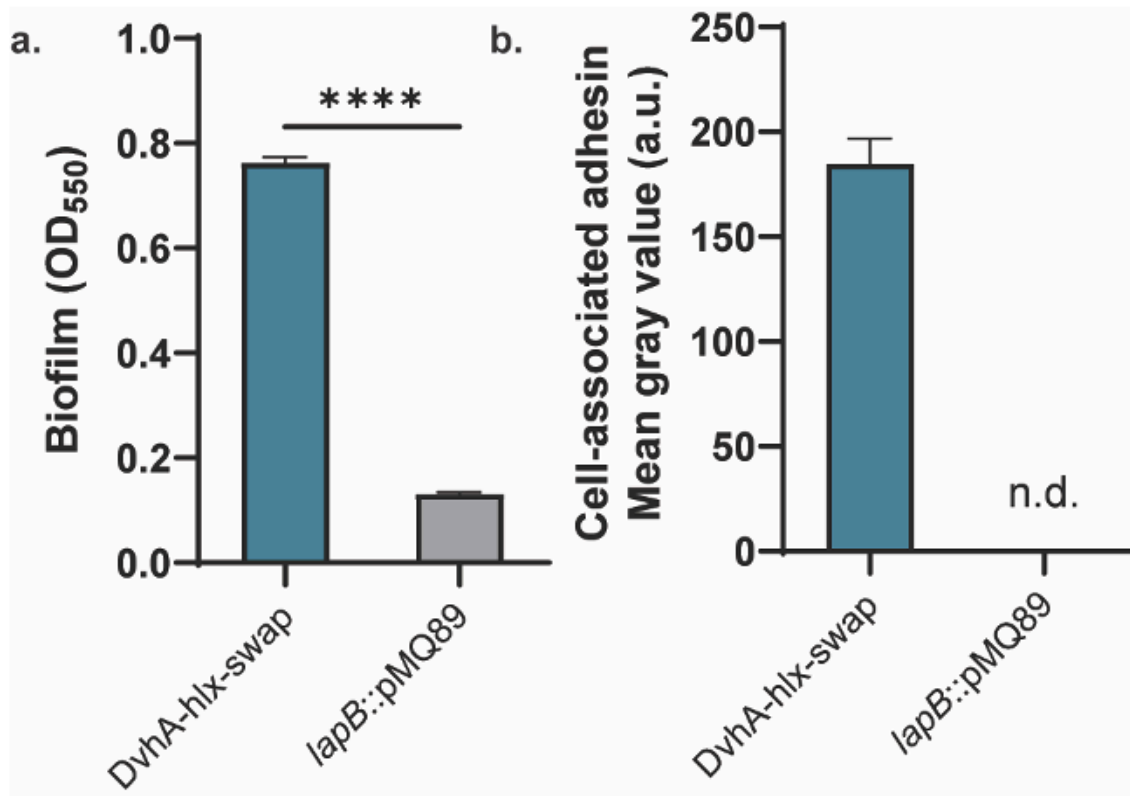


Figure S2: DvhA-hlx-swap used T1SS machinery of *P. fluorescens* Pf0-1. a) Biofilm formation in K10T-1 medium at 24 hours with DvhA-hlx-swap with and without the Pf0-1 T1SS ABC transporter component LapB. b) Quantification of cell-surface associated adhesin in both strains using dot blots. Signal intensity for lapB::pMQ89 was not detected (n.d.) Statistical analysis was performed using unpaired two-tailed t-test (ns, $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$).

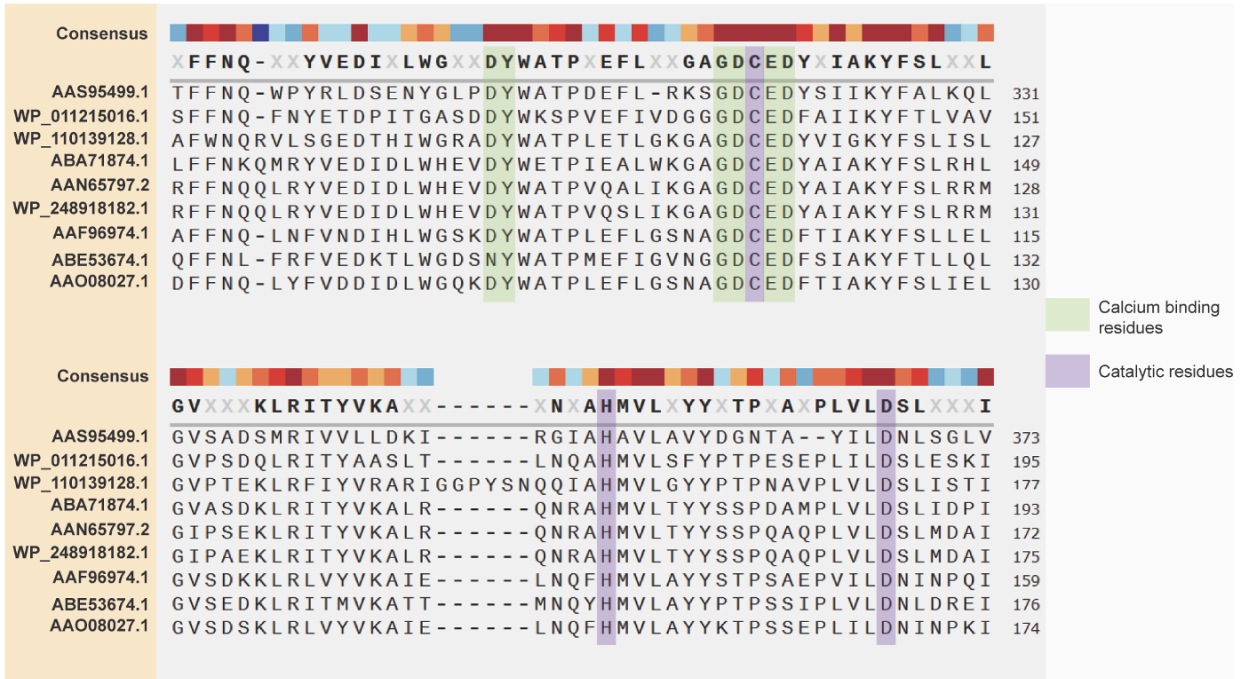


Figure S3: Multiple Sequence Alignment of DvhG with LapG-like proteases. Multiple sequence alignment of DvhG with LapG-like proteases using MUSCLE reveals conserved catalytic triad C-H-D (purple) and conserved calcium binding residues (green). The sequences used in the alignment are LapG-like proteases from *Desulfovibrio vulgaris* Hildenborough, *Legionella pneumophila*, *Bordetella bronchiseptica*, *Pseudomonas fluorescens* Pf0-1, *Pseudomonas putida* KT2440, *Pseudomonas entomophila*, *Vibrio cholerae* O1 biovar El Tor str N16961, *Shewanella denitrificans* OS217 and *Vibrio vulnificus* CMCP6, respectively.

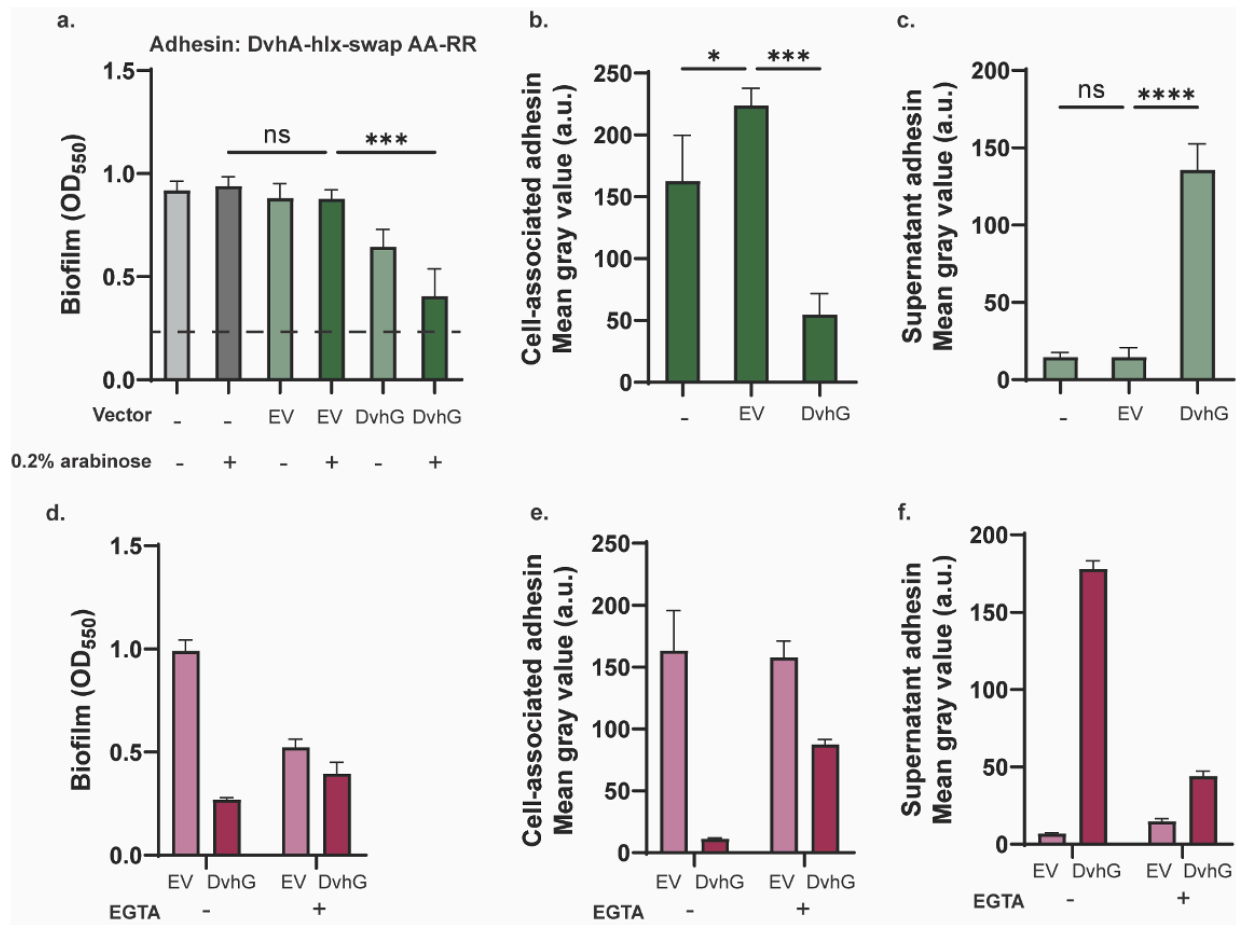


Figure S4: DvhG is a calcium ion dependent protease that targets dialanine in DvhA-hlx-swap. a) Biofilm formed in K10T-1 medium at 24 hours with *dvhA-hlx-swap* mutated at the dialanine site from 105PAAG108 to 105PRRG108 without a vector, with empty vector (EV) and *dvhG* expressed on an arabinose inducible plasmid in the presence or absence of arabinose. The dashed line represents the mean biofilm formed when DvhG processes the unmutated dialanine. b) Cell surface levels of DvhA-hlx-swap AA-RR in the presence of 0.2% arabinose. c) DvhA-hlx-swap AA-RR levels in the culture supernatants in the presence of 0.2% arabinose. d) Biofilm formed in K10T medium at 24 hours by the Pf0-1 $\Delta lapG \Delta lapD dvhA-hlx-swap$ strain with arabinose inducible empty vector (EV) or vector expressing *dvhG* in the presence of 0.2% arabinose, with or without 40 μ M EGTA (a calcium chelator). e) Quantification of cell-surface associated DvhA-hlx-swap with EV or *dvhG* induced with arabinose, with or without EGTA. f) DvhA-hlx-swap levels associated with the culture supernatants of the corresponding strains. Statistical analysis was performed using one way ANOVA multiple comparisons (ns, $p > 0.05$; *, $p \leq 0.01$; ***).

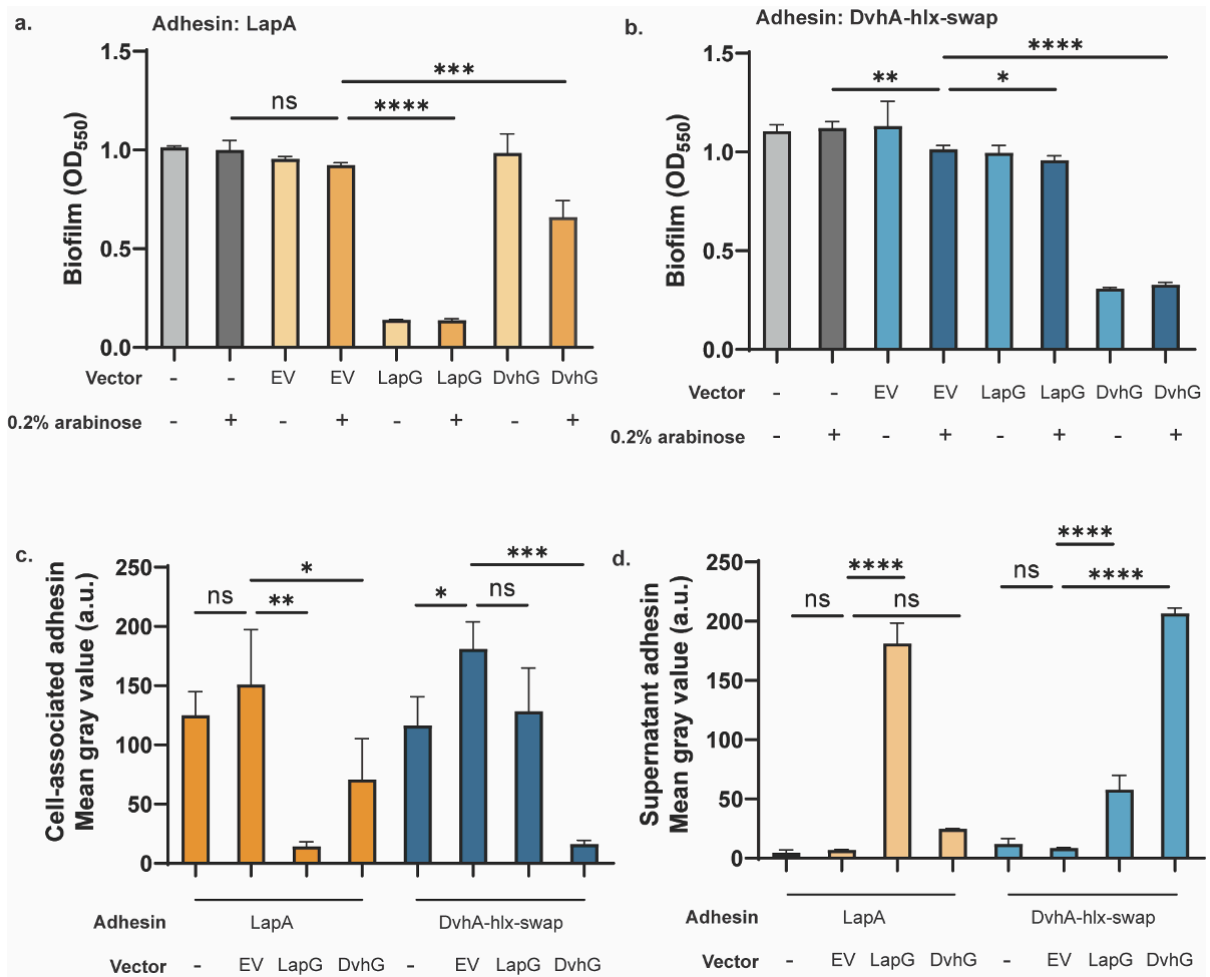


Figure S5. LapG and DvhG display specificity towards their native adhesins. a) Biofilm formation in K10T medium at 24 hours by the Pf0-1 $\Delta lapG \Delta lapD$ strain expressing the native adhesin LapA. Quantification was performed on the strains carrying no vector, arabinose inducible empty vector (EV), plasmid expressing *lapG* or *dvhG* in the presence or absence of arabinose. b) Biofilm formation in K10T medium at 24 hours by the Pf0-1 $\Delta lapG \Delta lapD$ strain expressing the fusion adhesin DvhA-hlx-swap. Quantification was performed on the strains carrying no vector, arabinose inducible empty vector (EV), plasmid expressing *lapG* or *dvhG* in the presence or absence of arabinose. c) Quantification of cell-surface associated adhesion of the strains in the presence of 0.2% arabinose. The bars in orange represent LapA and the bars in teal represent DvhA-hlx swap. d) Quantification of adhesin levels associated with the culture supernatants of the strains in the presence of 0.2% arabinose. The bars in coral represent LapA and the bars in light blue represent DvhA-hlx swap. Statistical analysis was performed using one way ANOVA multiple comparisons against the empty vector control (ns, $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$).

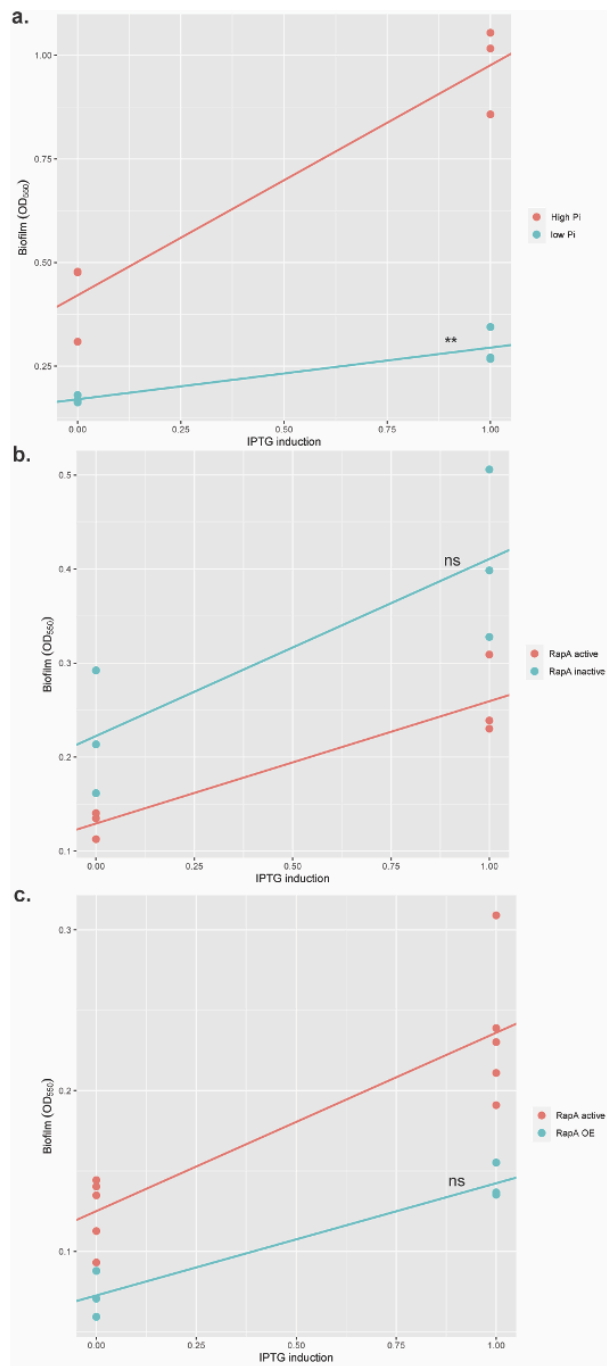


Figure S6: Statistical analysis to determine if biofilm rescue is dependent on c-di-GMP. Plots showing the effect of IPTG induction (0 – no IPTG, 1- 0.01% IPTG) on biofilm formation under a) high phosphate (red) vs low phosphate (blue) conditions. b) RapA-active (red) vs RapA-inactive (blue) strain under low phosphate conditions c) RapA-active (red) vs RapA overexpression (RapA OE, blue) strain under low phosphate conditions. Significance in all cases was calculated by linear regression with interaction term in R language (<https://github.com/GeiselBiofilm/>).

SI Text

Proteolytic activity of DvhG depends upon calcium ions.

Calcium has been shown to influence biofilm formation by bacteria (1, 2). For *P. fluorescens* Pf0-1, low calcium concentrations result in enhanced biofilm formation, as this lack of calcium negatively affects LapG's Ca-dependent activity and thus proteolysis of the N-terminus of LapA (3). The Multiple Sequence Alignment analysis also revealed conserved calcium-binding sites that could be critical for proteolysis (3, 4) (**Figure S3**).

To test if the activity of DvhG is dependent upon calcium ions, we added a calcium chelator (EGTA) to the K10T-1 medium to sequester extracellular calcium (5). Static biofilm assays were performed at 24 hours. We compared the biofilm formed by strain Pf0-1 $\Delta lapG \Delta lapD$ *dvhA-hlx-swap* expressing DvhG to the strain harboring the empty vector under conditions with and without 40 μ M EGTA, all in the presence of arabinose (**Figure S4d**). We found that EGTA compromised the ability of even our EV control to form biofilms. Upon calculating the percentage decrease in biofilm formation in the presence or absence of EGTA between the EV and DvhG, we find that this change is much smaller in the presence of EGTA (29%) than the absence (68%).

The dot blot analysis showed us a more convincing picture. Despite the observed reduction in biofilm formation +EGTA, EV controls under EGTA- and EGTA+ conditions were not significantly different ($p = 0.88$; **Figure S4e**), suggesting that the defect in biofilm formation in EV controls was due to a factor besides DvhG activity. For example, RTX proteins like LapA require Ca-binding to facilitate folding (6). Finally, we noted that 92% reduction in adhesin level on the cell surface when expressing DvhG without EGTA, but only 44% reduction with EGTA, implying the added EGTA reduced DvhG activity. The supernatant dot blots also complement these data (**Figure S4f**). Together, these data are consistent with the conclusion that calcium is required for the DvhG proteolytic activity.

LapG and DvhG display specificity towards their native adhesins.

To test whether LapG and DvhG can proteolyze their non-native adhesins, static biofilm assays were conducted at 24 hours comparing a plasmid carrying full length *lapG* (pMQ72-*lapG*), a plasmid carrying full length *dvhG* (pMQ72-*dvhG*) and an empty vector in two background strains: Pf0-1 $\Delta lapG \Delta lapD$ which expresses WT LapA adhesin and $\Delta lapG \Delta lapD$ *dvhA-hlx-swap* which expresses the fusion protein DvhA-hlx-swap. The plasmids were induced with 0.2% arabinose and compared to un-induced conditions.

Our results show that, when LapG is present, the strain expressing WT LapA has a significantly reduced ability to form a biofilm. With DvhG, on the other hand, the biofilm phenotype is significantly reduced as compared to the EV in the presence of arabinose ($OD_{550} = 0.66 \pm 0.08$ vs 0.98 ± 0.09 , $p = 0.01$), however it is also significantly higher than biofilms formed with a strain expressing LapG ($OD_{550} = 0.13 \pm 0.008$, $p = 0.0004$; **Figure S5a**).

When LapG is induced with arabinose in the strain expressing DvhA-hlx-swap, we observe a significant but modest loss in biofilm formation compared to the EV control ($OD_{550} = 0.95 \pm 0.02$ vs 1.01 ± 0.02). With DvhG expressed in this background, as expected, the loss of biofilm phenotype was more striking in comparison to the EV control (**Figure S5b**).

We calculated the percentage decrease in biofilm formation from the EV control with both proteases in arabinose-induced conditions. We noted that LapG caused an 85% decrease with LapA but only 6% decrease with DvhA-hlx-swap. DvhG caused a 68% decrease with DvhA-hlx-swap but only 30% decrease with LapA. Dot blot analysis suggests that changes in biofilm phenotype are due to changes adhesin levels on the cell surface, with a concomitant increase in the levels of adhesin in the supernatant (**Figure S5c-d**). These results suggest that these proteases are proficient in processing their own adhesins but less efficient towards the other adhesin.

Additional Materials and Methods

Static biofilm assays.

Pf0-1 strains were struck on LB agar plates overnight and a single colony was used to inoculate 5 ml LB for 16-18 hours of growth. 1.5 μ l of the LB overnight culture was used to inoculate 100 μ l of K10T-1 (or K10T π) medium in a 96-well round bottom polypropylene plates (Corning Life Sciences, Glendale, AZ). The plate was incubated in a humidified box at 30°C for 24 hours. The planktonic cells were discarded, and the plate was washed with water to remove any loosely bound cells. To stain the biofilms, 125 μ l crystal violet (0.1% (w/v)) was added to the wells and incubated at room temperature for 20 minutes. Excess stain was washed-off twice with water and the wells were allowed to dry before de-staining with 150 μ l solution of water, methanol, and glacial acetic acid (45:45:10) for 5 minutes. 100 μ l of the solubilized crystal violet from the wells was transferred to a 96-well flat bottom plate and the optical density was measured at 550 nm wavelength. Solubilized crystal violet from an uninoculated well was used as a blank for the measurements.

Quantitative surface adhesin localization assays using dot blots.

Pf0-1 strains expressing a WT 3x HA-tagged LapA or LapA variant were struck on LB agar plates overnight and a single colony was used to inoculate 5 ml LB for 16-18 hours. The LB overnight culture was used to inoculate 5 ml of K10T-1 medium (1:100) for growth at 30°C for 24 hours. The cultures were normalized to an OD₆₀₀ value of 0.5 and pelleted by centrifugation (13,200 rpm, 1 minute). The supernatants were collected, and filter sterilized using 0.22 µm syringe filters to probe for supernatant associated LapA. The pellets were washed with K10T-1 medium twice by resuspension and centrifugation. The pellets were finally resuspended in K10T-1 medium to an OD₆₀₀ of 0.5 to probe for cell-surface associated LapA. 5 µl of cell-surface associated and supernatant fractions were spotted on 0.2 µm nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA) and allowed to dry completely. The membranes were then incubated in a blocking solution containing 3% bovine serum albumin (BSA) in Tris-buffered saline with 1% Tween 20 (TBST) for 1 hour at room temperature. The blots were probed for LapA-HA using anti-HA antibody (BioLegend, San Diego, CA) at 1:2000 dilution in TBST containing 3% BSA overnight at 4°C. The membranes were washed thrice in TBST to remove excess antibody and incubated in horseradish peroxidase conjugated anti-mouse secondary antibody (1:15,000 dilution) in TBST for 1 hour at room temperature. Next, the membranes were washed twice in TBST and once in Tris-buffered saline (TBS) and incubated with Western Lightning Plus-ECL enhanced chemiluminescence substrate (PerkinElmer, Waltham, MA) for 1 minute. The membranes were imaged on a BioRad ChemiDoc MP Imaging System. Images were quantified using ImageJ software (NIH) as previously described in (7)

Detection and quantification of the proteins using Western blots.

Pf0-1 strains of interest were struck on LB agar plates (with antibiotics, if needed) overnight and a single colony was used to inoculate 5 ml LB for 16-18 hours of growth. The LB overnight culture was used to inoculate 5 ml of K10T-1 medium (1:100) at 30°C for 24 hours. DvhG overnights were subcultured (1:100) in 50 ml K10T-1 media. The cultures were OD₆₀₀ normalized and pelleted by centrifugation (13,200 rpm, 1 minute). The pellets were resuspended, lysed using French press and quantified for protein using Pierce™ BCA assay (ThermoFischer Scientific, Waltham, MA). The samples were boiled for 5 minutes in Laemmli sample buffer with 2-mercaptoethanol and were resolved on 7.5% (for adhesins) or 10% (for DvhD and DvhG) polyacrylamide gels. After transferring the proteins to a 0.2 µm nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA) and blocking with 3% BSA in TBST for 1 hour, the proteins were incubated in mouse anti-HA antibody in TBST (1:2000 dilution) overnight at 4°C for DvhD and adhesins. For DvhG, the blots

were incubated in Invitrogen mouse anti-His antibody at the same dilution (ThermoFischer Scientific, Waltham, MA). Fluorescently labelled anti-mouse secondary antibody was used to detect the protein bands on Odyssey Clx imager (LICOR Biosciences Inc., Lincoln, NE). The protein levels were quantified using Image Studio Lite software (LICOR Biosciences Inc., Lincoln, NE).

Cyclic-di-GMP quantification using flow cytometry. Strains harboring the c-di-GMP-dependent P_{CdrA} -*gfp* transcriptional reporter plasmid subcultured into K10T-1 media and incubated at 30°C for 24 hours. Strains without the plasmid were used as a non-fluorescent control to account for any background fluorescence. Cells were then washed, diluted, and analyzed on a Beckman 534 Coulter Cytoflex S as described previously in (8). FlowJo software version 10.8.1 was used to gate on populations of single cells. The GFP fluorescence from the P_{cdrA} promoter was then measured on the gated population. A workflow of the gating strategy is described in (8).

Supplementary Table 1: Strains and plasmids used in this study.

Strains	SMC Strain #	Description	Reference
<i>Escherichia coli</i>			
S17-1(λ -pir)	SMC117	<i>thi pro hsdR- hsdM+ ΔrecA</i> RP4-2::TcMu-Km::Tn7	(9)
<i>Pseudomonas fluorescens</i> Pf0-1			
$\Delta lapG\Delta lapD$	SMC 6422	Pf0-1 with unmarked deletion of <i>lapG</i> and <i>lapD</i>	(10)
$\Delta lapG\Delta lapD lapA$ -HA	SMC 5116	$\Delta lapG\Delta lapD$ with internal 3x HA epitope tag on <i>lapA</i>	(10)
$\Delta lapG\Delta lapD dvhA$ -RM-swap-HA	SMC 8159	$\Delta lapG\Delta lapD lapA$ -HA with N-terminal of <i>lapA</i> (aa 1-125) swapped with N-terminal of <i>dvhA</i> (aa 1-119) at the native locus.	This study
$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA	SMC 8176	$\Delta lapG\Delta lapD lapA$ -HA with the known <i>lapG</i> proteolysis residues of <i>lapA</i> (aa 81-123) swapped with predicted <i>dvhG</i> proteolysis residues of <i>dvhA</i> (aa 87-119) at the native locus.	This study
$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA AA-RR	SMC 9825	$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA with 105PAAG108 mutated to 105PRRG.	This study
$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA <i>lapB</i> ::pMQ89	SMC 9826	$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA with <i>lapB</i> gene disrupted on the <i>P. fluorescens</i> chromosome.	This study
$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA attTn7:: <i>lacI</i> -P _{tac} <i>dvhD</i> -HA	SMC 9827	$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA with <i>lacI</i> and <i>dvhD</i> -HA under P _{tac} promoter inserted at the <i>P. fluorescens</i> attTn7 site.	This study
$\Delta lapG\Delta lapD\Delta rapA$ <i>dvhA</i> -hlx-swap-HA attTn7:: <i>lacI</i> -P _{tac} <i>dvhD</i> -HA	SMC 9828	$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA attTn7:: <i>lacI</i> -P _{tac} <i>dvhD</i> -HA with unmarked <i>rapA</i> deletion.	This study
$\Delta lapG\Delta lapD$ P _{xutR} - <i>rapA</i> <i>dvhA</i> -hlx-	SMC 9829	$\Delta lapG\Delta lapD dvhA$ -hlx-swap-HA attTn7:: <i>lacI</i> -P _{tac} <i>dvhD</i> -HA with <i>rapA</i> under the control of xylose-inducible promoter P _{xutR} at its native locus	This study

<i>swap-HA attTn7:: lacI-P_{tac}dvhD-HA</i>			
Plasmids		Description	Reference
pMQ30	SMC 2765	Vector for cloning in yeast and allelic exchange in Gram-negatives; Gentamycin-resistance (Gm ^r) cassette	(11)
pMQ30- <i>dvhA-RM-swap</i>	SMC 8158	Vector to replace aa 1- 125 of <i>lapA</i> with aa 1-119 of <i>dvhA</i> using allelic exchange; Gm ^r	This study
pMQ30- <i>dvhA-hlx-swap</i>	SMC 8175	Vector to replace aa 81-123 of <i>lapA</i> with aa 87-119 of <i>dvhA</i> using allelic exchange; Gm ^r	This study
pMQ30- <i>dvhA-hlx-swap</i> AA-RR	SMC 9831	Vector to replace 105PAAG108 with 105PRRG108 in <i>dvhA-hlx-swap</i> using allelic exchange; Gm ^r	This study
pMQ56-mTn7	SMC 3242	Shuttle vector for insertion of the mini-Tn7 element from pUT18-mTn7-Gm into the attTn7 site on the <i>P. fluorescens</i> chromosome; Carbenicillin-resistance (Cb ^r) cassette	(11)
pBUX-BF13	SMC 1822	Helper plasmid encoding Tn7 transposition functions; Cb ^r	(12)
pFLP3	SMC 4060	Vector for removal of antibiotic resistance cassettes flanked by FRT sites with flipase; <i>sacB</i> for counterselection; Cb ^r	
pMQ56- <i>lacI</i> P _{tac} - <i>dvhD-HA</i>	SMC 9832	Vector for integration of <i>lacI</i> repressor and HA-tagged <i>dvhD</i> under IPTG-inducible Ptac promoter at the attTn7 site on the <i>P. fluorescens</i> chromosome; Cb ^r	This study
pMQ30- <i>rapA</i>	SMC 9833	Vector to produce an unmarked deletion of <i>rapA</i> from <i>P. fluorescens</i> chromosome by allelic exchange; Gm ^r	This study
pMQ72	SMC 2795	Expression vector for cloning in yeast, <i>E. coli</i> and <i>P. fluorescens</i> with arabinose-inducible gene expression system; Gm ^r	(11)
pMQ72- <i>dvhG-His</i>	SMC 9834	Vector for arabinose-inducible expression of full length <i>dvhG</i> with a 6x-His tag at the N-terminal; Gm ^r	This study
pMQ72- <i>dvhG</i> Δ <i>TM-His</i>	SMC 9835	Vector for arabinose-inducible expression of <i>dvhG</i> without aa 1-40 and with a 6x-His tag at the N-terminal; Gm ^r	This study

pMQ72- <i>dvhG</i> -His C317A	SMC 9836	Vector for arabinose-inducible expression of full length <i>dvhG</i> -C317A with a 6x-His tag at the N-terminal; Gm ^r	This study
pMQ72- <i>lapG</i>	SMC 5143	Vector for arabinose-inducible expression of full length <i>lapG</i> ; Gm ^r	(10)
pMQ89	SMC 2952	Suicide vector to generate single-crossover constructs in <i>P. fluorescens</i> chromosome; Gm ^r	(11)
pMQ89- <i>lapB</i>	SMC 5148	Vector to introduce disruption in <i>lapB</i> gene on <i>P. fluorescens</i> chromosome; Gm ^r	(13)
pMQ650	SMC 9396	Expression vector for cloning with xylose-inducible gene expression system; Gm ^r	(14)
pCdrA-gfpC	SMC 5709	Vector for fluorescence-based quantification of cyclic di-GMP in <i>Pseudomonas aeruginosa</i> and related species	(15)

Supplementary Table 2: Oligonucleotides used in this study.

Oligonucleotide Name	Sequence (5' → 3')
DvhA_RMswap_up_F	GTCGACTCTAGAGGATCCCCCGCTGTCGGTGCCTTCAC
DvhA_RMswap_up_R	GATGGTGCGATTGAGAGGCATTGAAGACTCTCCGGGTGTCAC
DvhA_RMswap_down_F	GCAGGTAGCGGCGGTGCCGCTGGTGGCGGC
DvhA_RMswap_down_R	CGAATTCGAGCTCGGTACCCCCTTCGGTCACGCTTGG
DvhA_RMinsert_F	GTGACACCCGGAGAGTCTTCAATGCCTCTCAATCGCACCATC
DvhA_RMinsert_R	GTCGCATCGAGCATCACGAAACCGCCGCTACCTGC
DvhA_hlxswap_up_F	GTCGACTCTAGAGGATCCCCCGCTGTCGGTGCCTTCAC
DvhA_hlxswap_up_R	CCGGGGAGTTCCTGACCGGTGGCTTCGGCCAGGTC
DvhA_hlxswap_down_F	GCAGGTAGCGGCGGT ACTGGCGGTGCCGCT
DvhA_hlxswap_down_R	CGAATTCGAGCTCGGTACCCCCTTCGGTCACGCTTGG
DvhA_hlxInsert_F	GACCTGGCCGAAGCCACCGGTCAGGAACTCCCCGG
DvhA_hlxInsert_R	CCAGCGGCACCGCCACCGCCGCTACCTGC
DvhG_nHis_F	TCGAGCTCGGTACCCGAAGGAGATATACATATGCATCATCACCATCAC CACGCAAAGGCGCGGGAGA
DvhG_R	GACTCTAGAGGATCCCCTCATTTCCTCCCGGCTTTCTTC
DvhG-noTM_nHis_F	TCGAGCTCGGTACCCGAAGGAGATATACATATGCATCATCACCATCAC CACGGAGATACCGAAGCTGGGG
delrapA_up_F	GTCGACTCTAGAGGATCCCCCGGCGAGCGATCCGTTC
delrapA_up_R	GTTTCGTGCATTTGAAGAGGGGCAATCTCTGGCGATAAAAAAAGG
delrapA_down_F	ATCGCCAGAGATTGCCCTCTTCAAATGCACGAAACGGC
delrapA_down_R	CGAATTCGAGCTCGGTACCCGGGCGGAAGTGAAATTTGATAAACG
rapAup_F	GTCGACTCTAGAGGATCCCCACGTAGTCCGGCCGC
rapAup_R	CCAAGAACAACAAGGAGGATTTTATGACCACGACCGAACAG
PxutR_insert_F	GCTGTTTCGGTTCGTGGTCATAAAATCCTCCTTGTTGTTCTTGG
PxutR_insert_R	ATCGCCAGAGATTGCCCGAGCCCTATCGGCTGG
rapAdown_F	CCAGCCGATAGGGCTCGGGCAATCTCTGGCGATAAAAAAAGG
rapAdown_R	CGAATTCGAGCTCGGTACCCCGGCGAGCGATCCGTTC
LapB_seqF	GGCCGAGATCAAAAACATTCAGTTAT
LapB_seqR	TGGCCCACAGCATCAGG
Fra1_Ptaclacl- dvhD_mTn7_F	CTCACTAGTGGATCCCCCTCACTGCCCGCTTTCCAGTC
Fra1_Ptaclacl-dvhD _mTn7_R	ACAGGAAACAGACTAGTGCTCTGCAGAAGGAGATATACATATGTACCCATAC

Fra2_Ptaclacl-dvhD _mTn7_F	ACAGGAAACAGACTAGTGCTCTGCAGAAGGAGATATACATATGTACCCATAC
Fra2_Ptaclacl-dvhD _mTn7_R	GGAATTCCTGCAGCCCTCAGCCTTGACGCACATCAAGC
hlxswap_RR_up_F	GTCGACTCTAGAGGATCCCCGGCAACCGGAATGACCGTATG
hlxswap_RR_up_R	CGGCGGTTCGCATCGAGCAT
hlxswap_RR_down_F	ATGCTCGATGCGACCGCCG
hlxswap_RR_down_R	CGAATTCGAGCTCGGTACCCGATGGTGTTCGGTGACCTGAGTC

References

1. M. J. Arrizubieta, A. Toledo-Arana, B. Amorena, J. R. Penadés, I. Lasa, Calcium inhibits Bap-dependent multicellular behavior in *Staphylococcus aureus*. *J. Bacteriol.* **186**, 7490–7498 (2004).
2. S. Theunissen, *et al.*, The 285 kDa Bap/RTX hybrid cell surface protein (SO4317) of *Shewanella oneidensis* MR-1 is a key mediator of biofilm formation. *Res. Microbiol.* **161**, 144–152 (2010).
3. C. D. Boyd, D. Chatterjee, H. Sondermann, G. A. O’Toole, LapG, required for modulating biofilm formation by *Pseudomonas fluorescens* Pf0-1, is a calcium-dependent protease. *J. Bacteriol.* **194**, 4406–4414 (2012).
4. D. Chatterjee, C. D. Boyd, G. A. O’Toole, H. Sondermann, Structural characterization of a conserved, calcium-dependent periplasmic protease from *Legionella pneumophila*. *J. Bacteriol.* **194**, 4415–4425 (2012).
5. R. Y. Tsien, New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis, and properties of prototype structures. *Biochemistry* **19**, 2396–2404 (1980).
6. A. Chenal, J. I. Guijarro, B. Raynal, M. Delepierre, D. Ladant, RTX calcium binding motifs are intrinsically disordered in the absence of calcium: implication for protein secretion. *J. Biol. Chem.* **284**, 1781–1789 (2009).
7. A. J. Collins, A. B. Pastora, T. J. Smith, G. A. O’Toole, MapA, a second large RTX adhesin conserved across the pseudomonads, contributes to biofilm formation by *Pseudomonas fluorescens*. *J. Bacteriol.* **202** e00277–20 (2020).
8. C. J. Geiger, G. A. O’Toole, Evidence for the Type IV pilus retraction motor PilT as a component of the surface sensing system in *Pseudomonas aeruginosa*. *J. Bacteriol.* **205**, e00179–23 (2023).
9. R. Simon, U. Priefer, A. Pühler, A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. *BioTechnology* **1**, 784–791 (1983).
10. P. D. Newell, C. D. Boyd, H. Sondermann, G. A. O’Toole, A c-di-GMP effector system controls cell adhesion by inside-out signaling and surface protein cleavage. *PLoS Biol.* **9**, e1000587 (2011).
11. R. M. Q. Shanks, N. C. Caiazza, S. M. Hinsa, C. M. Toutain, G. A. O’Toole, *Saccharomyces cerevisiae*-based molecular tool kit for manipulation of genes from gram-negative bacteria. *Appl. Environ. Microbiol.* **72**, 5027–5036 (2006).
12. Y. Bao, D. P. Lies, H. Fu, G. P. Roberts, An improved Tn7-based system for the single-copy insertion of cloned genes into chromosomes of gram-negative bacteria. *Gene* **109**, 167–168 (1991).

13. R. D. Monds, P. D. Newell, R. H. Gross, G. A. O'Toole, Phosphate-dependent modulation of c-di-GMP levels regulates *Pseudomonas fluorescens* Pf0-1 biofilm formation by controlling secretion of the adhesin LapA. *Mol. Microbiol* **63**, 656-679 (2007).
14. J. D. Callaghan, *et al.*, Xylose-inducible promoter tools for *Pseudomonas* species and their use in implicating a role for the Type II secretion system protein XcpQ in the inhibition of corneal epithelial wound closure. *Appl. Environ. Microbiol.* **86**, e00250-20 (2020).
15. M. T. Rybtke, *et al.*, Fluorescence-based reporter for gauging cyclic Di-GMP levels in *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* **78**, 5060–5069 (2012).