Supporting Information

Petrova and Sauer 10.1073/pnas.1207832109

SI Materials and Methods

Bacterial Strains and Growth Conditions. All bacterial strains and plasmids used in this study are listed in Table S3. *P. aeruginosa* strain PAO1 was used as parental strain. *Pseudomonas aeruginosa* planktonic cultures were grown in LB or VBMM minimal medium (1) in shake flasks at 220 rpm in the absence or presence of 1.0% arabinose. *Escherichia coli* cultures were grown in LB in the absence or presence of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). Antibiotics were used at the following concentrations: 50–75 μg/mL gentamicin and 200–250 μg/mL carbenicillin for *P. aeruginosa*; and 20 μg/mL gentamicin and 50 μg/mL ampicillin for *E. coli*.

Strain Construction. Complementation and overexpression of full-length *bdlA*, *clpD* (PA0459), *clpP, PA4843*, and *dipA* or truncated *bdlA* constructs was accomplished by placing the respective genes under the control of an arabinose-inducible promoter in the pJN105 (2) or pMJT-1 (3) vectors. C-terminal V5/His-tagging was accomplished by subcloning into pET101D (Invitrogen), and a C-terminal HA tag was introduced into the indicated genes via PCR using the sequence agcgtagtctgggacgtcgtatgggta. The tagged constructs were introduced into pJN105 and pMJT1. V5/His-tagged *bdlA* under control of its native promoter (−500 bp relative to the translational start site) was also cloned into the mini-CTX vector (4). Site-directed mutagenesis of indicated *bdlA* sequences was accomplished by using the GeneArt Site-Directed Mutagenesis Kit (Invitrogen) according to the manufacturer's protocol. Plasmids were introduced into *P. aeruginosa* via conjugation or electroporation. Primers used for strain construction are listed in Table S4.

Biofilm Formation. Biofilms were grown using a once-through continuous-flow tube reactor system for dispersion assays and to obtain protein samples, and in flow cells to view the biofilm architecture before and after induction of dispersion as previously described (5–9). Biofilms were grown at 22 °C in 1/20 diluted LB or VBMM minimal medium in the absence/presence of 0.1% arabinose. Antibiotics were used in tube reactors at the following concentrations: 2 μg/mL gentamicin and 10 μg/mL carbenicillin. Quantitative analysis of CSLM images of flow cell-grown biofilms was performed using COMSTAT (10).

Biofilm Dispersion. Biofilm dispersion assays were performed as previously described (11). In brief, following five days of biofilm growth in continuous-flow tube reactors, biofilm dispersion was induced by a sudden increase of the carbon concentration in the growth medium, by the addition of 19.8 mM glutamate. In subsequence, the optical density at 600 nm of the biofilm effluent, collected in 1-min intervals, was measured, with dispersion events indicated by an increase in the turbidity of the effluent. In addition, dispersion was visualized by confocal microscopy and the response subsequently quantitated by COMSTAT analysis as previously described (12).

Immunoblot Analysis and Pull-Downs. Abundance and processing of tagged BdlA constructs was assessed by SDS/PAGE and immunoblotting using anti-V5 antibodies. Pull-down assays were used to assess the interactions between various truncated BdlA constructs and PA0459. Total protein cell extracts containing HA-tagged bait proteins, as indicated, were incubated with extracts containing V5/His-tagged prey proteins, as indicated. Subsequently, HA-tagged proteins were immunoprecipitated using immobilized anti-HA antibodies, immunoprecipitation eluates separated by SDS/PAGE, and assessed by immunoblot analysis for the presence of V5/His-tagged prey proteins using anti–V5-HRP antibodies. Pull-down assays were carried out using 200 μg protein from cellular extracts. The indicated antibodies (Invitrogen Corp) were used for immunoprecipitation at 1 μg/mL and immunoblotting at 0.1 μg/mL.

Identification of BdlA Protein Interaction Partners. The protein interaction partners of BdlA were identified using two-dimensional polyacrylamide gel electrophoresis (2D/PAGE) analysis of immunoprecipitation reactions of V5/His-tagged BdlA. In brief, the equivalent of 1.5 mg of total cell protein extracts of PAO1/pJN-*bdlA*-V5/His were immunoprecipitated using 5 μg of anti-V5 antibodies, with PAO1/pJN105 used as a control. The immunoprecipitation eluates were subjected to separation by 2D/PAGE, using pH3-10NL immobilized pH gradient (IPG) strips, with the resulting gels analyzed using the ImageMaster analysis software (GE Healthcare). Protein spots determined to copurify specifically with BdlA-V5/His were identified by liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described (7, 9, 13).

Protein Cross-Linking. Complex formation between various BdlA domains was confirmed via reversible in vivo cross-linking with DSP. In brief, following incubation for 9 or 19 min with 2 mM DSP (stock solution prepared at 15 mM in DMSO), cells containing indicated BdlA constructs were centrifuged for 1 min at 16,000 × *g*, for total cross-linking times of 10 and 20 min, respectively. The cross-linking reactions were stopped by resuspending and lysing the cells via sonication directly into TE buffer [10 mM Tris·HCl (pH 8.0), 1 mM EDTA, plus 0.3 μg/mL PMSF]. Cross-linking was reversed using β-mercaptoethanol (β-ME), and cross-linked samples were treated using a nonreducing SDS/PAGE loading buffer.

Statistical Analysis. A Student's *t* test was performed for pairwise comparisons of groups, and multivariant analyses were performed using a one-way ANOVA followed by a postpriori test using Sigma Stat software. All experiments were carried out at least in triplicate.

1. Schweizer HP (1991) The *agmR* gene, an environmentally responsive gene, complements defective *glpR*, which encodes the putative activator for glycerol metabolism in *Pseudomonas aeruginosa*. *J Bacteriol* 173:6798–6806.
2. Newman JR, Fuqua C (1999) Broad-host-range expression vectors that carry the L-arabinose-inducible Escherichia coli araBAD promoter and the araC regulator. *Gene* 227:197–203.
3. Kaneko Y, Thoendel M, Olakanmi O, Britigan BE, Singh PK (2007) The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. *J Clin Invest* 117:877–888.
4. Hoang TT, Kutchma AJ, Becher A, Schweizer HP (2000) Integration-proficient plasmids for *Pseudomonas aeruginosa*: Site-specific integration and use for engineering of reporter and expression strains. *Plasmid* 43:59–72.
5. Allegrucci M, et al. (2006) Phenotypic characterization of *Streptococcus pneumoniae* biofilm development. *J Bacteriol* 188:2325–2335.
6. Allegrucci M, Sauer K (2007) Characterization of colony morphology variants isolated from *Streptococcus pneumoniae* biofilms. *J Bacteriol* 189:2030–2038.
7. Petrova OE, Sauer K (2009) A novel signaling network essential for regulating *Pseudomonas aeruginosa* biofilm development. *PLoS Pathog* 5:e1000668.
8. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG (2002) *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J Bacteriol* 184:1140–1154.
9. Southey-Pillig CJ, Davies DG, Sauer K (2005) Characterization of temporal protein production in *Pseudomonas aeruginosa* biofilms. *J Bacteriol* 187:8114–8126.
10. Heydorn A, et al. (2000) Quantification of biofilm structures by the novel computer program COMSTAT. *Microbiology* 146:2395–2407.
11. Sauer K, et al. (2004) Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. *J Bacteriol* 186:7312–7326.
12. Morgan R, Kohn S, Hwang S-H, Hassett DJ, Sauer K (2006) BdlA, a chemotaxis regulator essential for biofilm dispersion in *Pseudomonas aeruginosa*. *J Bacteriol* 188:7335–7343.
13. Petrova OE, Sauer K (2010) The novel two-component regulatory system BfiSR regulates biofilm development by controlling the small RNA rsmZ through CafA. *J Bacteriol* 192:5275–5288.

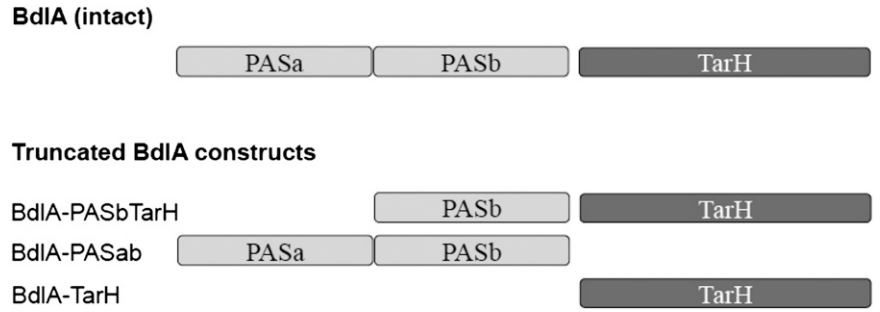

Fig. S1. Overview of domain composition of intact and truncated BdlA constructs used in this study.

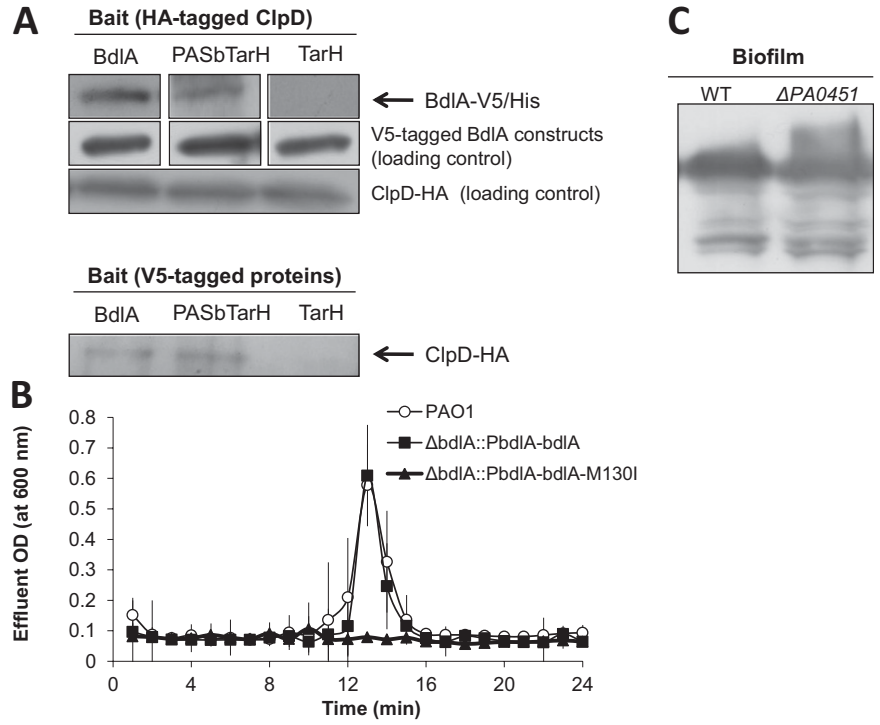

Fig. S2. Immunoblot analysis demonstrating complex formation and the role of BdlA-M130I and putative protease PA0451 in dispersion and BdlA processing. (*A*) Immunoblot analysis of in vivo pull-down assays demonstrating that complex formation between ClpD and BdlA depends primarily on PASa. Pull-downs were performed using both V5-tagged BdlA constructs and HA-tagged ClpD as bait. Loading controls are shown. (*B*) Complementation of Δ*bdlA* with the site-directed BdlA-M130I mutant protein does not restore the biofilm dispersion phenotype of Δ*bdlA* to wild-type levels. Biofilms were grown in tube reactors for 5 d before dispersion was induced by the sudden addition of glutamate. *P. aeruginosa* PAO1 and Δ*bdlA* were used as controls. (*C*) Inactivation of the putative protease PA0451 does not impair BdlA processing under biofilm growth conditions. Wild-type and ΔPA0451 mutant biofilms were grown in tube reactors for 5 d. Immunoblots were probed with anti-V5 antibodies.

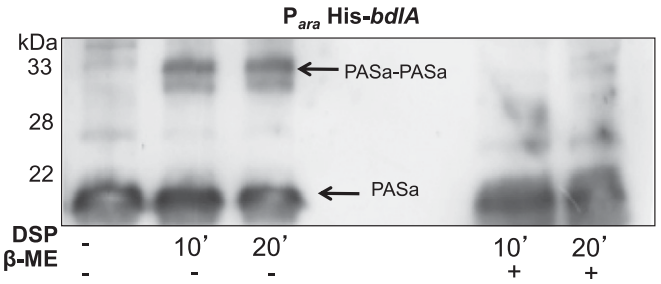

Fig. S3. Dimerization of BdlA PASa domains. PASa interacts with PASa in PAO1/pJN-His-*bdlA*(P_{BAD}) as indicated using in vivo cross-linking and probing with anti-His antibodies. Cross-linking with DSP was reversed using β-ME.

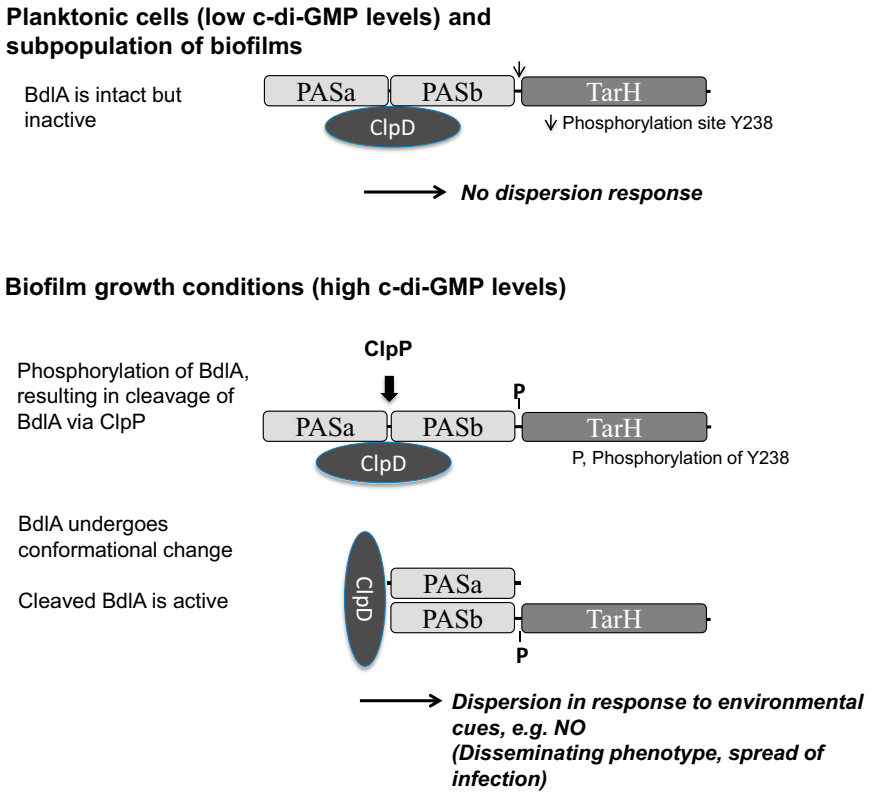

Fig. S4. Proposed model of BdlA activation to induce dispersion upon exposure to environmental cues. Dispersion by *P. aeruginosa* biofilms requires post-translationally modified BdlA. Under planktonic growth conditions and within a subpopulation of biofilm cells, BdlA is present in its intact form and not phosphorylated. Intact BdlA is inactive with respect to enabling the dispersion response. Under biofilm growth conditions, BdlA is phopshorylated, with phopshorylation at tyrosine 238 (Y238) likely being a signal for BdlA cleavage. The nonprocessive cleavage is likely carried out by a ClpP-like protease. Although ClpD is required for BdlA cleavage, ClpD interacts with BdlA independently of the posttranslational modifications of BdlA via its PAS domains indicating that ClpD may have a stabilizing function with respect to BdlA. Following cleavage, the PASa and PASb domains of BdlA interact, which results in conformational change of the processed BdlA protein. Such posttranslationally modified BdlA enables dispersion in response to environmental cues such as nitric oxide (NO) or changes in the nutrient levels. Dispersion enables bacteria to leave the biofilm and return to the planktonic mode of growth. The status of BdlA and its effect on dispersion may be linked to a switch in the virulence phenotype of *P. aeruginosa*.

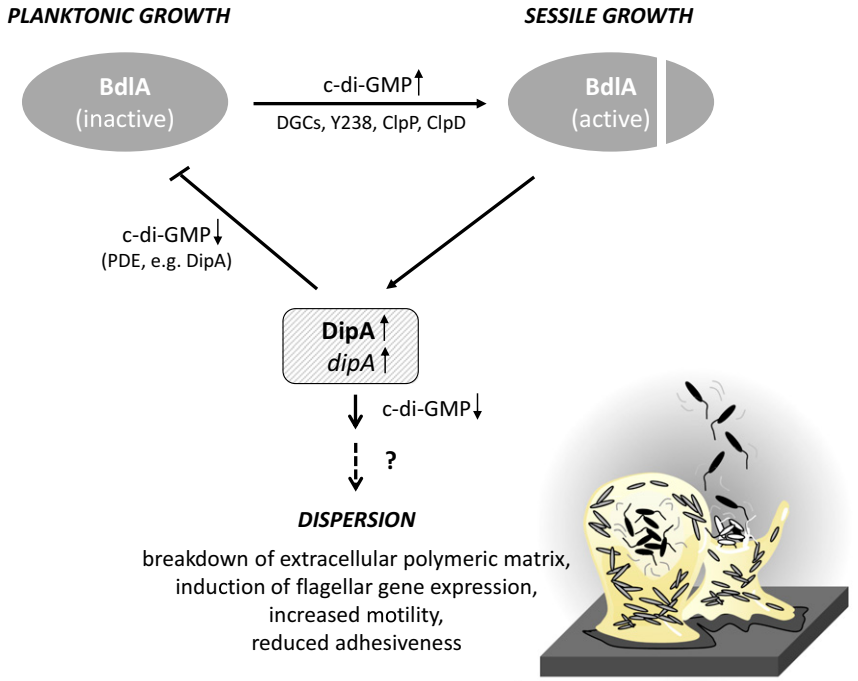

Fig. S5. Proposed model of the role of BdlA in *P. aeruginosa* biofilm dispersion. Under planktonic conditions, BdlA is intact but inactive. Upon perceiving elevated levels of c-di-GMP following transition to surface-associated growth, BdlA is cleaved in a nonprocessive manner, a process requiring the chaperone ClpD, the protease ClpP, and BdlA phosphorylation at Y238. Cleaved BdlA is active with respect to enabling *P. aeruginosa* biofilms to respond to dispersion inducing conditions. BdlA and DipA were recently demonstrated to interact in vivo (1). Moreover, dispersion correlated with increased interaction of BdlA with the phosphodiesterase DipA under biofilm growth conditions, resulting in increased DipA levels, increased phosphodiesterase activity, and reduced biofilm biomass accumulation (1). Although it has been well demonstrated that increased DipA activity results in decreased biofilm c-di-GMP levels (2), it is not fully understood how DipA activity and reduced c-di-GMP levels contribute to dispersion (see dashed arrow). Regardless of the mechanism, dispersion has been described to require or coincide with the breakdown of extracellular polymeric matrix surrounding the biofilms, induction of flagellar gene expression, increased motility, and reduced adhesiveness (3–6). Reduced c-di-GMP levels furthermore impair BdlA activation. Dashed arrow and question mark indicate that the connection has not been fully demonstrated or is not understood at the molecular level. DGC, diguanulyte cyclase.

1. Petrova OE, Sauer K (2012) PAS domain residues and prosthetic group involved in BdlA-dependent dispersion response by *Pseudomonas aeruginosa* biofilms. *J Bacteriol*.
2. Roy AB, Petrova OE, Sauer K (2012) The phosphodiesterase DipA (PA5017) is essential for *Pseudomonas aeruginosa* biofilm dispersion. *J Bacteriol* 194:2904–2915.
3. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG (2002) *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J Bacteriol* 184:1140–1154.
4. Sauer K, et al. (2004) Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. *J Bacteriol* 186:7312–7326.
5. Morgan R, Kohn S, Hwang S-H, Hassett DJ, Sauer K (2006) BdlA, a chemotaxis regulator essential for biofilm dispersion in *Pseudomonas aeruginosa*. *J Bacteriol* 188:7335–7343.
6. Newman JR, Fuqua C (1999) Broad-host-range expression vectors that carry the L-arabinose-inducible Escherichia coli araBAD promoter and the araC regulator. *Gene* 227:197–203.

Table S1. Detection of posttranslational modifications present in BdlA

Modification/position*	Peptide sequence†
Acetylation (K226)	VV<u>K</u>FASDVSDR
Phosphorylation (Y238)	<u>Y</u>QAEADNAHQAHTLSTETR

*Type of posttranslational modification and position of modified amino acid in the BdlA protein sequence.
†Peptide sequence and posttranslational modification was determined using ProID and MASCOT following MS/MS analysis of tryptic peptides of BdlA. Modified amino acid is underlined.

Table S2. Identification of BdlA residues and proteins essential for BdlA proteolysis and dispersion

P. aeruginosa strains	Dispersion response*	Biofilm biomass†	
		Before dispersion	Post dispersion
PAO1	+	12.8 (8.1)	1.2 (1.6)
Δ*bdlA*	–	11.7 (5.4)	11.3 (6.9)
Δ*bdlA*/*bdlA*	+	11.7 (6.9)	2.8 (1.9)
Δ*clpD*	–	6.0 (2.6)	6.5 (2.9)
Δ*bdlA*/*bdlA*-M130I	–	6.9 (4.0)	6.5 (3.7)
Δ*bdlA*/*bdlA*-Y238A	–	7.7 (3.9)	7.9 (3.9)

+, dispersion detected; –, no dispersion detected.

*Dispersion in response to glutamate was determined using the biofilm tube reactor dispersion method.

†Visualization and quantitation of the biofilm biomass before and following induction of dispersion by glutamate was done by confocal microscopy and subsequent COMSTAT analysis.

Table S3. Bacterial strains and plasmids

Strains/plasmids	Relevant genotype or description	Source
Strains		
E. coli		
DH5-α	F-φ80*lacZ*ΔM15 *Δ(lacZYA-argF)*U169 *rec*A1 *end*A1 *hsd*R17 *(r_k^-, m_k^+) pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 *tonA*	Invitrogen Corp.
BL21	F-*ompT hsdS*$_B$ ($r_B^-m_B^-$) *gal dcm* (DE3)	Invitrogen Corp
P. aeruginosa		
PAO1	Wild type	B. H. Holloway, Monash University, Clayton, VIC, Australia
ΔbdlA	*ΔbdlA* in PAO1; Km^R	(1, 2)
PAO1/pJN-*bdlA*-HA	Arabinose-inducible expression of HA-tagged *bdlA*; Gm^R	This study
PAO1/pJN-*bdlA*-V5/6xHis	Arabinose-inducible expression of V5/6xHis-tagged *bdlA*; Gm^R	This study
PAO1/pJN-*bdlA*-PASbTarH-HA	Arabinose-inducible expression of HA-tagged PASbTar *bdlA*; Gm^R	This study
PAO1/pJN-*bdlA*-TarH-HA	Arabinose-inducible expression of HA-tagged *bdlA*-TarH; Gm^R	This study
PAO1/pJN-*bdlA*-NoTarH-HA	Arabinose-inducible expression of HA-tagged *bdlA*-NoTarH; Gm^R	This study
PAO1/P_{bdlA}-*bdlA*-V5/His	Expression of *bdlA* under native *bdlA* promoter, at *attB*	This study
ΔbdlA/P_{bdlA}-*bdlA*-V5/His	Chromosomal complementation of *ΔbdlA* under native *bdlA* promoter, at *attB*	This study
ΔbdlA/pJN-*bdlA*-V5/His	Complementation of *ΔbdlA* under P_{BAD} promoter	This study
ΔbdlA/pJN-PASbTarH	Complementation of *ΔbdlA* with PASb-TarH domains of BdlA, cloned into pJN105	This study
ΔbdlA/pJN-PASab	Complementation of *ΔbdlA* with PASab domains of BdlA	This study
ΔbdlA/pJN-TarH	Complementation of *ΔbdlA* with TarH domain of BdlA	This study
ΔbdlA/pJN-PASa/pMJT-*bdlA*-PASbTarH	Complementation of *ΔbdlA* with separately encoded PASa and PASbTarH domains of BdlA	This study
ΔbdlA/P_{bdlA}-*bdlA*-Y238A	Chromosomal complementation of *ΔbdlA* with BdlA-Y238A	This study
ΔbdlA/P_{bdlA}-*bdlA*-M130I	Chromosomal complementation of *ΔbdlA* with BdlA-M130I	This study
ΔclpD/P_{bdlA}-*bdlA-V5/His*	PAO1 PA0459::IS*lacZ*; Expression of *bdlA* under native *bdlA* promoter at *attB*, Tet^R	This study
ΔclpD/ P_{bdlA}-*bdlA*-V5/His /pJN-*clpD*	Complementation of *ΔclpD*/P_{bdlA}-*bdlA-V5/His*; Gm^R	This study
ΔclpP/P_{bdlA}-*bdlA*-V5/His	*clpP*::IS*lacZ*; Expression of *bdlA* under native *bdlA* promoter, at *attB, Tet*^{R}	This study
ΔclpP/P_{bdlA}-*bdlA*-V5/His/pJN-*clpP*	Complementation of *ΔclpP*/P_{bdlA}-*bdlA-V5/His*; *Tet*^{R}, Gm^R	This study
ΔPA0451//P_{bdlA}-*bdlA*-V5/His	PA0451::IS*lacZ*; Expression of *bdlA* under native *bdlA* promoter, at *attB, Tet*^{R}	This study
Plasmids		
pCR2.1-TOPO	TA cloning vector; Km^R; Ap^R	Invitrogen Corp.
pRK2013	Helper plasmid for triparental mating; *mob; tra*; Km^R	(3)
pJN105	Arabinose-inducible gene expression vector; pBRR-1 MCS; *ara*C-P_{BAD}; Gm^R	(4)
mini-CTX-*lacZ*	Integration vector for single-copy, chromosomal *lacZ* fusions; Ω-*FRT*-*attP*-MCS, *ori, int,* and *oriT*; Tet^R	(5)
pET101D	Vector for directional cloning and high level V5/6XHis fusion protein expression, Amp^R	Invitrogen Corp
pMJT1	*ara*C-P_{BAD} cassette of pJN105 cloned into pUCP18, Amp^R ($Carb^R$)	(6)
pET-*bdlA*-V5/6xHis	*bdlA* cloned into pET101D	This study
pET-P_{bdlA}-*bdlA*-V5/6xHis	*bdlA* cloned into pET101D with native promoter	This study
pET-PASa	PASa of *bdlA* cloned into pET101D	This study
pET-PASab	PASab of *bdlA* cloned into pET101D	This study
CTX-PbdlA-*bdlA*-V5/6xHis	V5-6xHis-tagged *bdlA* with native promoter cloned into mini-CTX	This study
CTX-PbdlA-*bdlA*-M130I	CTX-PbdlA-*bdlA*-V5/6xHis with an M130I mutation in *bdlA*	This study
CTX-PbdlA-*bdlA*-Y238A	CTX-PbdlA-*bdlA*-V5/6xHis with an Y238A mutation in *bdlA*	This study
pJN-*bdlA*-HA	C-terminal HA-tagged bdlA cloned into pJN105 at NheI/SacI	This study
pJN-*bdlA*-V5/6xHis	C-terminal V5/6xHis-tagged *bdlA* cloned into pJN105 at *EcoR*I/SpeI	This study
pJN-PASa	PASa-encoding segment of *bdlA* cloned into pJN105 at *EcoR*I/SacI	This study
pJN-*bdlA*-PASbTarH-HA	C-terminal HA-tagged PASaTar of *bdlA* cloned into pJN105 at NheI/SacI	This study
pJN-*bdlA-TarH*-HA	C-terminal HA-tagged Tar of *bdlA* cloned into pJN105 at NheI/SacI	This study
pJN-*bdlA*-PASab-HA	HA-tagged PASab of *bdlA* cloned into pJN105 at NheI/SacI	This study
pJN-*bdlA*-V5/6xHis	C-terminal V5/6xHis-tagged bdlA cloned into pJN105 at *EcoR*I/SpeI	This study
pJN-*bdlA*-PASbTarH-V5/6xHis	C-terminal V5/6xHis-tagged PASaTar of *bdlA* cloned into pJN105 at NheI/SpeI	This study
pMJT-*bdlA*-PASbTarH	C-terminal V5/6xHis-tagged PASaTar of *bdlA* cloned into MJT-1 at NheI/SpeI	This study
pJN-*bdlA*-TarH-V5/6xHis	C-terminal V5/6xHis-tagged Tar of *bdlA* cloned into pJN105 at NheI/SpeI	This study
pJN-*bdlA*-PASab-V5/6xHis	C-terminal V5/6xHis-tagged PASab of *bdlA* cloned into pJN105 at *EcoR*I/SpeI	This study
pJN-*clpP*	clpP cloned into pJN105	This study
pJN-*clpD*	HA-tagged *clpD* cloned into pJN105 at NheI/SacI	This study

1. Morgan R, Kohn S, Hwang S-H, Hassett DJ, Sauer K (2006) BdlA, a chemotaxis regulator essential for biofilm dispersion in *Pseudomonas aeruginosa*. *J Bacteriol* 188:7335–7343.
2. Kulasakara H, et al. (2006) Analysis of *Pseudomonas aeruginosa* diguanylate cyclases and phosphodiesterases reveals a role for bis-(3′-5′)-cyclic-GMP in virulence. *Proc Natl Acad Sci USA* 103:2839–2844.
3. Figurski DH, Helinski DR (1979) Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided *in trans*. *Proc Natl Acad Sci USA* 76:1648–1652.
4. Newman JR, Fuqua C (1999) Broad-host-range expression vectors that carry the L-arabinose-inducible Escherichia coli araBAD promoter and the araC regulator. *Gene* 227:197–203.
5. Becher A, Schweizer HP (2000) Integration-proficient *Pseudomonas aeruginosa* vectors for isolation of single-copy chromosomal *lacZ* and *lux* gene fusions. *Biotechniques* 29:948–950, 952.
6. Kaneko Y, Thoendel M, Olakanmi O, Britigan BE, Singh PK (2007) The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. *J Clin Invest* 117:877–888.

Table S4. Oligonucleotides

Oligonucleotide	Sequence*
Directional cloning into pET101D	
bdlA_pET_for	CACCATGGCGGCCCTGGACC
P_{bdlA}_pET_for	caccCTCGGCGAAACGCTCGCC
bdlA_pET_rev	GAGATCGGCGTTGAGGGTGC
PASab_pET_rev	CATGCGGTCGCTGACATCGC
PASa_pET_rev	CATCTCGTGTTCCTGGTGGAC
Reverse primers for C-term V5/6xHis-tagged constructs out of pET101D	
pETHis_rev_EcoRI	GCGCGCgaattcTCAATGGTGATGGTGATG
pETHis_XbaI_rev	GCGCGCtctagaTCAATGGTGATGGTGATG
General cloning	
bdlAf_His_Eco	gaattcATGGCGGCCCTGGACCG
bdlA_NheI_for	GCGCGCGCgctagcATGGCGGCCCTGGACCG
P_{bdlA}HisV5_EcoRI_for	GCGCGCGCgaattcCTCGGCGAAACGCTCGCC
bdlA_NoPAS1_NheI_for	GCGCGCgctagcATGGAGCAAGCTGGATGCCC
bdlA_TarH_only_NheI_for	GCGCGCGCgctagcATGAGCGATGTCAGCGACCGC
clpD-NheI-for	GCGCGCgctagcGTGGACATCGCCGAATAC
clpP_NheI_for	GCGCGCGCgctagcCGCAAGACATGTCTCGCAAC
clpP_SacI_rev	GCGCGCGCgagctcGTATTGCGGAGCCTTAGACG
bdlAPAS1_SacI_rev	GCGCGCGCgagctcCTGCATCTCGTGTTCCTGGTGGAC
HA-tagging†	
bdlA_HA_SacI_rev	GCGCGCGCgagctcCTAagcgtagtctgggacgtcgtatgggtaGAGATCGGCGTTGAGGGTGCGCG
bdlA_NoTarH_HA_SacI_rev	GCGCGCGCgagctcCTAagcgtagtctgggacgtcgtatgggtaGCGGTCGCTGACATCGCT
clpD_HA_SacI_rev	GCGCGCGAGCTCCTAagcgtagtctgggacgtcgtatgggtaCTTGGCCTTCGGCTTGTC
Site-directed mutagenesis	
Y238A-for	CAGCGACCGCATGCGCCGCGCCCAGGCCGAGGCGGACAAC
Y238A-rev	GTTGTCCGCCTCGGCCTGGGCGCGGCGCATGCGGTCGCTG
M130I-for	GGATGCCCTGTCCCGCTCGATCGCGATGATCGAGTTCGACCTC
M130I-rev	GAGGTCGAACTCGATCATCGCGATCGAGCGGGACAGGGCATCC

*Restriction sites are indicated by nucleotides in lowercase.
†Underlined sequence in lowercase denotes sequence for HA tag.