Supporting Online Material for

A Virulence Locus of *Pseudomonas aeruginosa* Encodes a Protein Secretion Apparatus

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Material and Methods

Strain and plasmid construction

P. aeruginosa strains used in this study were all derivatives of the sequenced strain PAO1 (*S1*). *Escherichia coli* strain DH5α was used for cloning, and *E. coli* strain Sm10l*pir* was used to mate plasmids into *P. aeruginosa*. In-frame deletions and chromosomal fusions were performed by allelic replacement using the pEXG2 suicide plasmid (*S2*). In-frame gene deletions were constructed using splicing by overlap extension (SOE) PCR reactions (*S3*). Primers were designed such that each deletion resulted in the replacement the entire open reading frame, with the exception of first two codons and the penultimate codon with the sequence 5'-TTCAGCATGCTTGCGGCTCGAGTTCCA-3'. This leads to an open reading frame encoding a peptide with the sequence: N-X_1X_2FSMLAARVPYX_3-C, where X_{1-3} are the first, second, and penultimate codons of the targeted ORF, respectively. To construct a chromosomal fusion of the VSV-G epitope to *hcp1* (PA0085), 500 bp regions flanking the C-terminus of *hcp1* were amplified. The sequence 5'-TATACAGATATTGAAATGAATAGATTAGGAAAATGA -3' was used to replace the stop codon of *hcp1*. The chromosomal fusion of *clpV1* (PA0090) to *gfp* was also generated using the allelic replacement vector pEXG2; however, the stop codon of *clpV1* was replaced with the sequence encoding *gfp(mut3)* using a SpeI linker site (this introduces Thr-Ser linkage between the last amino acid of ClpV1 and the start codon of GFP). To complement Δ*clpV1* in the Δ*retS* background, the sequence encoding *clpV1*, including the putative ribosome binding site (RBS) was amplified by PCR and cloned into pPSV35 downstream of the *lacUV5* promoter (*S2*). The resulting complementation construct was electroporated into electrocompetent Δ*retS* Δ*clpV1*. Complementation of Δ*clpB* (PA4542) was achieved using similar methods.

Immunoblot analysis

One milliliter of overnight cultures of *P. aeruginosa* cultures grown in Luria Broth (LB) medium were washed once with 1 mL LB medium; 3 μL of washed cultures were added to 2 mL of LB medium and grown until early log phase. 1.5 mL samples were harvested and pelleted at 9000 x g for 3 min. One milliliter of the supernatant was removed and centrifuged for 4 min at 10000 x g; 750 μL of the resulting supernatant was added to 85 μL of ice-cold trichloroacetic acid (TCA). TCA precipitation was allowed to proceed for 5 min on ice before a 15 min centrifugation at 16000 x g. The supernatant was removed, and precipitated proteins were washed once with acetone and then resuspended in 25 μL final sample buffer. Cell fractions were prepared by removing the remaining culture supernatants and resuspending cell pellets in 50 μL final sample buffer. Supernatant and cell pellet samples were boiled for 5 min, centrifuged for 2 min at 16000 x g, and 10 μL samples were loaded onto 8-16% pre-cast polyacrylamide gels (Biorad). Proteins were transferred to nitrocellulose membranes. Membranes were blocked using 5% non-fat milk in Tris buffered saline containing 0.1% Tween and probed simultaneously with a rabbit polyclonal anti-VSV-G antibody (1:2000, Sigma) and a mouse anti-RNA polymerase β-subunit antibody (1:2000, Neoclone) or blots of clinical isolate samples

were probed with a rabbit anti-Hcp1 antibody (1:2000, in-house). Blots were washed twice with TBST, probed with an anti-rabbit and/or anti-mouse horseradish peroxidase-conjugated secondary antibodies (1:5000, Sigma), and washed two additional times with TBST. The resulting blots were incubated for 5 min in ECL reagent and detected using O-MAT X-ray film (Kodak). Blots analyzed for GFP and ClpV1-GFP expression were treated similarly, but were probed with anti-GFP antibody (1:2000, Sigma). For sputum analysis, 10 μL of liquefied CF patient sputum was analyzed by anti-Hcp1 immunoblot as described above with the exception that the secondary antibody was used at 1:100,000 and the signal was detected with ultrasensitive ECL reagent (Pierce).

α-Hcp1 Titer Analysis

Adult subjects and parents of those less than 18 years of age signed consents approved by the Committee on Clinical Investigation at Children's Hospital, Boston. His-Select 96 well microtiter plates (Sigma) were loaded with 0.5 μg/well of purified His-tagged Hcp1 or 0.5 μg of a purified His-tagged cytosolic *P. aeruginosa* control protein (PilS). Two-fold serum dilutions (starting at 1:50) were prepared in Tris-buffered saline with 1% w/v BSA. After washes, binding was detected with anti-human IgA + IgG + IgM (H+L) conjugated to alkaline phosphatase (Pierce) by measuring 4-nitrophenyl phosphate hydrolysis at 405 nm. Serum from two CF patients that were not exposed to *P. aeruginosa* were used as naive controls. Endpoint titer was determined where the measurement exceeded two-fold above background ($absorbance_{405nm}$ of > 0.10).

Thermotolerance assays

P. aeruginosa strains were grown at 37°C to an $OD_{600\ nm}$ of 0.1 in LB medium supplemented with 30 μg/mL gentamicin and 0.25 mM isopropyl-β-D-thiogalactoside. At this point, an aliquot of each culture was removed and the number of colony forming units was determined by plating serial dilutions on LB agar. The remaining culture was placed in a 55°C water bath for 25 min. Following the heat pulse, cells were placed on ice, and colony forming units were again determined by plating serial dilutions on LB agar.

Fluorescence microscopy and flow cytometry

P. aeruginosa strains analyzed by fluorescent microscopy were back-diluted 1:1000 from overnight cultures and grown to mid-log phase in baffled flasks with shaking at 37°C. A 1 mL aliquot of this culture was removed and the cells were centrifuged for 1 min at 8000 x g. The supernatant was aspirated, and the cells were resuspended in 10 μL of phosphate buffered saline containing 0.01 mM of the lipophylic membrane dye TMA-DPH (Molecular Probes). Fluorescence microscopy was performed with an Olympus BX61 microscope as previously described (*S4*). Fluorescent signals were visualized with a phase contrast objective UplanF1100× and captured with a monochrome CoolSnapHQ digital camera (Photometrics) using Metamorph software version 6.1 (Universal Imaging). Exposure times were 100 ms for TMA-DPH visualization and 200 ms for GFP visualization. Images were analyzed, adjusted and cropped using Metamorph software. All images presented were treated identically so that a direct visual comparison of expression levels can be made.

Cultures used for flow cytometry experiments were prepared identically to those used in fluorescence microscopy experiments. The cells were analyzed with a FACScalibur flow cytometer (Becton Dickinson).

Hcp1 expression and purification

The *hcp1* (PA0085) open reading frame was cloned into the pMCSG7 expression vector, which introduces an N-terminal fusion of the protein to a His_6-tag and a TEV protease recognition site (*S5*, *S6*). The fusion protein was over-produced in a *Bacillus cereus* BL21-derivative harboring a plasmid encoding three rare tRNAs (Arg [AGG/AGA] and Ile [ATA]). Cells were grown at 37°C in a defined M9 selenomethionine (SeMet) growth media in the presence of 50 ug/ml Ampicillin and 30 ug/ml Kanamycin as described earlier (*S7*). The cells were induced with 1 mM isopropyl-a-D-thiogalactoside (IPTG) when the optical density at 600 nm reached 0.8, and were incubated at 20°C for overnight, and then were harvested and suspended in 50 mM HEPES(Na)/HCL at pH=8.0, 500 mM NaCL,10 mM imidazole, 5% glycerol, 10 mM β-mercaptoethanol; and lysed by lysozyme and sonication. The fusion Se-Met protein was purified by affinity chromatography using His-Trap HP (GE Health Systems). The His_6-tag was removed by the rTEV digestion and Ni-NTA (Qiagen). The protein was concentrated with simultaneous buffer exchange using Centricon plus-20 (Amicon) (5-kD cutoff). The molecular weight of Hcp1 in solution was determined by size-exclusion chromatography on a Superdex–200 10/30 column (Pharmacia) calibrated by ribonuclease A (13.7 kD), ovalbumin (43 kD), albumin (67 kD), aldolase (158 kD), catalase (232 kD), and thyroglobulin (669 kD) as standards.

Electron microscopy and image processing

The Hcp1 sample was diluted to a final concentration of 0.01 mg/ml in a solution containing 10 mM Tris-HCl pH 7.5 and 0.5 M NaCl, and negatively stained with uranyl formate as described (*S8*). Electron micrographs were recorded with an FEI Tecnai T12 electron microscope at an acceleration voltage of 120 kV. Images were taken at a magnification of 52,000x and a defocus of –1.5 μm using low-dose conditions. Images were taken on Kodak SO-163 film and developed for 10 minutes with full-strength Kodak D-19 developer at 20˚C. Electron micrographs were digitized with a SCAI scanner (Zeiss) using a step size of 7 μm. 2 x 2 pixels were averaged yielding a pixel size of 2.7 Å on the specimen level. We interactively selected 5,456 Hcp1 particles from 14 micrographs using WEB, the display program associated with the SPIDER software package (*S9*), which was used for further image processing. The selected particles were windowed into of 64 x 64 pixel images and subjected to ten cycles of multi-reference alignment and classification specifying 50 output classes. Representative classes are shown in Fig. 4D.

Hcp1 Structure Determination

A 44 mg/ml protein stock solution in 20 mM HEPES at pH=8.0, 200 mM NaCl and 1 mM DTT was used for crystallization. The best crystals were obtained using vapor diffusion and hanging droplets from 100 mM Tri-Sodium citrate dehydrate, 100 mM HEPES buffer pH= 7.5 and 16 % w/v Polyethylene Glycol 3350 at 22°C. Crystals

appeared in 2 days and reached a maximum size in 4 days. A single crystal was cryo-protected by the removal of excess mother liquor in Paritone N and then flash frozen in liquid nitrogen. X-ray diffraction analysis indicated the crystal belonged to space group P6 with cell dimensions 146.7Å x 146.7Å x 42.2Å and diffracted beyond 1.95 Å Bragg spacings. Multiwavelength anomalous dispersion (MAD) data were collected at 105 K at the Structural Biology Center 19ID beamline at the Advanced Photon Source of Argonne National Laboratory. Peak and inflection point energies were based on the X-ray absorption spectrum of the SeMet-crystal and the remote was 100eV above the peak. Data collection strategy, integration, and scaling were performed with the HKL2000 package (*S10*). Data set details can be found in Table S3.

Eighteen initial Se positions were found with the heavy search algorithm in the CNS suite v1.1 (*S11*). These sites were subsequently used to search and optimize 21 sites in autoSHARP (*S12*), in which phasing and solvent modification was also performed. Model-building programs O (*S13*) and COOT (*S14*) were to produce a complete monomer (chain A), which was then docked into the remaining two members of the asymmetric unit (chains B and C). The model was subject to iterations of refinement, manual correction, and solvent addition until a final R/R-free=17.9/22.7% was achieved. TLS (each monomer defined as a group) and restrained refinement was performed with REFMAC against peak data to 1.95 Å and Hendrickson-Lattmann coefficients from autoSHARP prior to density modification (*S15*). No non-crystallographic restraints were imposed during refinement. The final model contains three Hcp1 monomers and 551 water molecules, with the following residues missing due to poor electron density: A-2 to A1, A46, B-2 to B1, B43 to B47, and C-2 to C1, inclusive. All residues fell in acceptable areas of a Ramachandran plot, with 93.8% in the most favored region and 3.2% in the additional allowed regions as determined by PROCHECK (*S16*). Atomic coordinates have been deposited in the Protein Data Bank (http://www.rcsb.org) under accession code 1Y12.

All molecular graphic figures were generated using Pymol (http://pymol.sourceforge.net). The electrostatic surface of Hcp1 was generated based on calculated vacuum electrostatics. The sequence conservation scores of Hcp1 residues were generated using Consurf (http://consurf.tau.ac.il). The Hcp1 homologs used to generate the multiple sequence alignment were downloaded directly using the Hcp1 Blink option at the NCBI webpage (http://www.ncbi.nlm.nih.gov). These sequences were filtered for identical and redundant proteins, and proteins from unclassified organisms before being aligned using ClustalW (*S17*).

References

S1. C. K. Stover *et al.*, *Nature* **406**, 959 (2000).
S2. A. Rietsch, I. Vallet-Gely, S. L. Dove, J. J. Mekalanos, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 8006 (2005).
S3. R. M. Horton *et al.*, *Methods Enzymol.* **217**, 270 (1993).
S4. D. Z. Rudner, Q. Pan, R. M. Losick, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 8701 (2002).
S5. L. Stols *et al.*, *Protein Expr. Purif.* **25**, 8 (2002).
S6. L. Dieckman, M. Gu, L. Stols, M. I. Donnelly, F. R. Collart, *Protein Expr. Purif.* **25**, 1 (2002).
S7. M. A. Walsh, I. Dementieva, G. Evans, R. Sanishvili, A. Joachimiak, *Acta Crystallogr. D Biol. Crystallogr.* **55 (Pt 6)**, 1168 (1999).
S8. M. Ohi, Y. Li, Y. Cheng, T. Walz, *Biol. Proced. Online* **6**, 23 (2004).
S9. J. Frank *et al.*, *J. Struct. Biol.* **116**, 190 (1996).
S10. Z. Otwinowski, W. Minor, *Methods Enzymol.* **276**, 307 (1997).
S11. A. T. Brunger *et al.*, *Acta Crystallogr. D Biol. Crystallogr.* **54 (Pt 5)**, 905 (1998).
S12. G. Bricogne, C. Vonrhein, C. Flensburg, M. Schiltz, W. Paciorek, *Acta Crystallogr. D Biol. Crystallogr.* **59**, 2023 (2003).
S13. T. A. Jones, J. Y. Zou, S. W. Cowan, Kjeldgaard, *Acta Crystallogr. A* **47 (Pt 2)**, 110 (1991).
S14. P. Emsley, K. Cowtan, *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126 (2004).
S15. M. D. Winn, M. N. Isupov, G. N. Murshudov, *Acta Crystallogr. D Biol. Crystallogr.* **57**, 122 (2001).
S16. R. A. Laskowski, M. W. MacArthur, D. S. Moss, J. M. Thornton, *J. Appl. Cryst.* **26**, 283 (1993).
S17. J. D. Thompson, D. G. Higgins, T. J. Gibson, *Comput. Appl. Biosci.* **10**, 19 (1994).

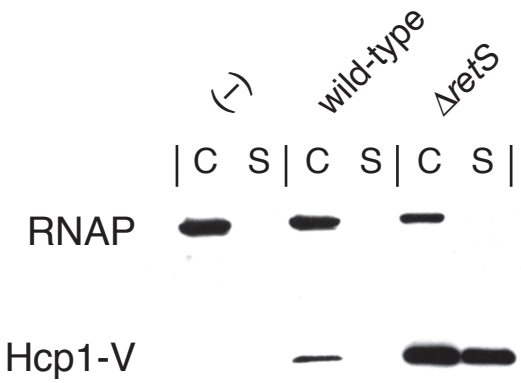

Fig. S1. Hcp1 secretion is repressed by RetS. Western blot analysis showing qualitative differences in Hcp1-V secretion levels between the wild-type and ΔretS backgrounds containing the *hcp1-V* chromosomal fusion. Samples in the (-) lanes were prepared from PAO1 lacking the *hcp1-V* fusion. In order to visualize Hcp1 in wild-type cells, 5-fold more sample was loaded than in the Δ*retS* lanes and the blot exposure was increased relative to Fig. 2B. Hcp1-V was not detected in the supernatant fraction of wild-type cells.

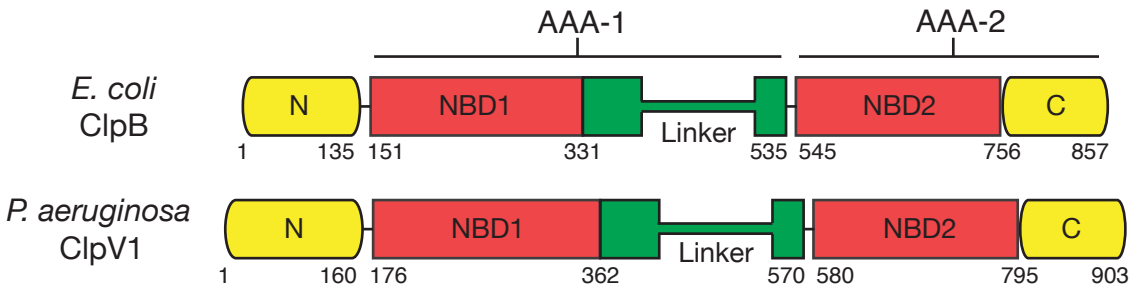

Fig. S2. Comparison of the domain organization of *E. coli* ClpB and that predicted for *P. aeruginosa* ClpV1. The ClpB domain boundaries and functions are based on their prior assignment from structural studies of the protein. N- and C-terminal domains are shown in yellow, nucleotide-binding domains (NBD) in red and the ClpB/Hsp104-specific linker in green. The narrow region of the linker domain represents an extended coiled-coil. The proteins are 35% identical overall and 45% identical within the NB and linker domains.

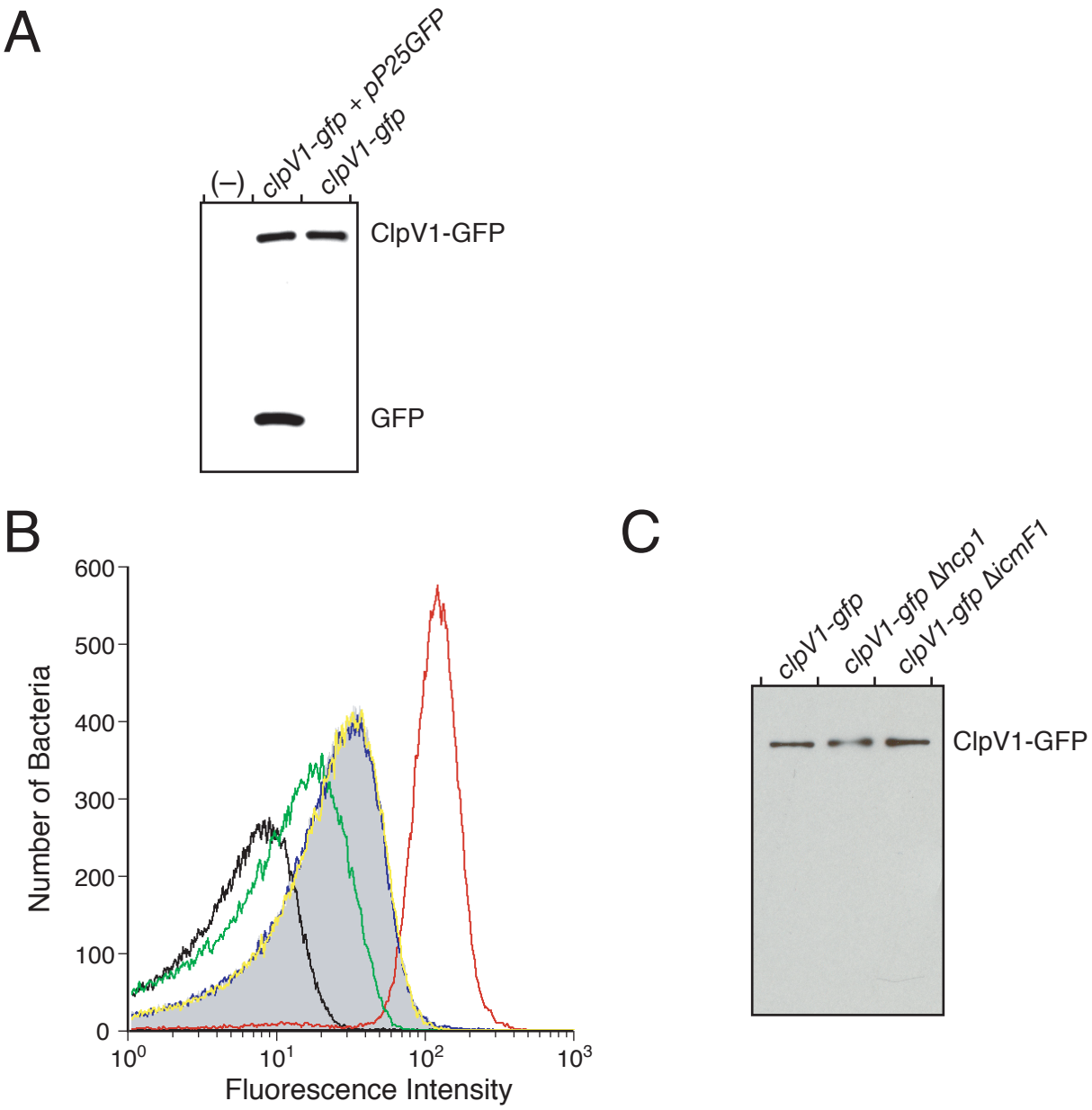

Fig. S3. The stability and expression of ClpV1-GFP are unaffected by deletion of *hcp1* or *icmF*. (A) α-GFP Western blot analysis of whole cell extracts of *P. aeruginosa* wild-type (-), Δ*retS clpV1-gfp,* and Δ*retS clpV1-gfp* expressing GFP (pP25GFP) strains. No free GFP is detected in Δ*retS clpV1-gfp.* (B) Flow cytometry analysis comparing GFP expression in Δ *retS clpV1-gfp* (grey fill) to isogenic strains bearing additional mutations in *icmF1* (yellow) and *hcp1* (blue). *clpV1-gfp* in the wild-type background is shown in green. Wild-type (black) and wild-type containing a plasmid expressing GFP (pP25GFP, red) are included as controls. (C) α-GFP Western blot analysis of whole cell extracts of the indicated *P. aeruginosa* strains. No free GFP is detected in Δ*retS clpV1-gfp* or derivative strains with HSI-I gene deletions.

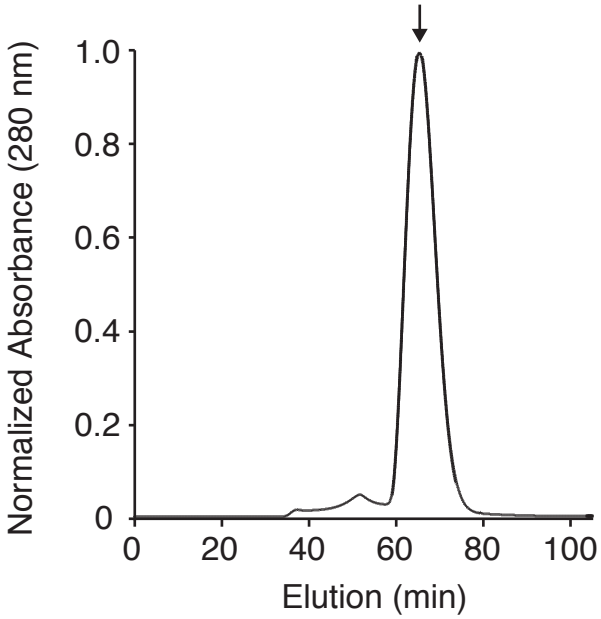

Fig. S4. Gel filtration chromatograph of purified Hcp1. This analysis yielded a measured mass of 80 kDa for Hcp1, consistent with a large oligomeric species. The arrow indicates the position at which Hcp1 was withdrawn for electron microscopic analysis (Fig. 4D).

Table S1. Subset of genes in IAHP-related loci of selected Gram-negative pathogens.

COG #[1]	COG description	Comments	*Pseudomonas aeruginosa* (PAO1)			*Pseudomonas syringae*[2] (DC3000)	*Vibrio cholerae* (E1 Tor 16961)	*Vibrio vulnificus* (CMCP6)	*Salmonella typhimurium* (LT2)	*Agrobacterium tumefaciens* (ATCC 33970)	*Escherichia coli* O157:H7 (Sakai)	*Yersinia pestis*[2] (KIM5)	*Yersinia pseudotuberculosis* (IP32953)	*Burkholderia pseudomallei*[2] (1710b)	*Bordetella parapertussis* BAA-587
			HSI-I	HSI-II	HSI-III										
3523	Function unknown	Similar to type IV secretion factor, IcmF3[3]	*icmF1* (PA0077)	PA1669	PA2361	PSPTO2554	*icmF* (VCA0120)	VV20441	*sciS*[4,5] (STM0285)	*icmF* (AGR_L_1061)	Ecs0218	*icmF3* (y3658)	YPTB3625	BPse171_ 01004292	BPP0730
0542	ATPase, chaperone activity	Highly similar to ClpB[3]	*clpV1*[3] (PA0090)	PA1662	PA2371	PSPTO2548	VCA0116	VV20430	*sciG*[4] (STM0272)	AGR_L_1041	Ecs0223	*htpM* (y3669)	*clpB2* (YPTB3629)	BPse171_ 01004282	BPP0724
3157	Function unknown	Hemolysin coregulated protein (Hcp)[6]	*hcp1* (PA0085)	PA1512[7]	PA2367	*hcp1* (PSPTO2539)	*hcp-2*[7] (VCA0017[7])	VV20429	*sciK*[4] (STM0276)	AGR_L_1037	Ecs0216	*vgrG* (y1034)	YPTB3639	BPSe171_ 01004286	BPP0716
3501	Function unknown	Rhs element Vgr protein[6]	PA0091	PA1511[7]	PA2373	PSTPO2538	*vgrG-2*[7] (VCA0018) *vgrG-3* (VCA0123)	VV20248	*vrgS*[4] (STM0289)	*vgrG* (AGR_L_1033)	Ecs0236	y3659	YPTB3623	BPSe171_ 0100481	-
3455	Function unknown	OmpA family, similar to type IV secretion factor DotU[3, 8]	PA0078	PA1668	-	PSTPO2553	VCA0115	VV20442	*sciP*[4] (STM0282)	AGR_L_1060	Ecs0224	-	YPTB3630	BPSe171_ 0100493	BPP0729
0515	Ser/thr protein kinase	Ser/thr protein kinase[9,10]	*ppkA* (PA0075)	PA1671	-	-	-	VV20446	-	AGR_L_1065	-	-	-	BPSe171_ 01004290	BPP0732
0631	Ser/thr protein phosphatase	Protein phosphatase 2C-like[9]	PA0074	*stp1* (PA1670)	-	-	-	VV20439	-	-	-	-	-	-	-
3456	Function unknown, forkhead associated domain (FHA)	Putative ser/thr kinase substrate[3,11]	PA0081	PA1665	-	-	VCA0112	VV20445	-	AGR_L_1057	Ecs0227	-	YPTB3633	BPSe171_ 01004297	-

[1]Cluster of Orthologous Groups (COG) designated for each gene (source: Integrated Microbial Genomes interface (http://img.jgi.doe.gov)) discussed and annotated in current manuscript

[2]Multiple IAHP-related loci present in this organism. For brevity, only one locus is represented here.

[3]Originally defined as IAHP constituents in Das, S and Chaudhuri, K. *In Silico Biol.* 2003;3(3):287-300.

[4]Folkesson A. *et al. Res. Microbiol.* 2002 Oct;153(8):537-45.

[5]Parsons and Heffron. *Infect. Immun.* 2005 Jul;73(7):4338-45.

[6]Pukatzki *et al*. *Proc. Natl. Acad. Sci. U S A.* 2006 Jan 31;103(5):1528-33.

[7]Genes reside outside of the cluster, but demonstrated (*V. cholerae* Hcp-2, VgrG-2 and VgrG-3) or predicted (*P. aeruginosa* PA1512) to function with proteins encoded by genes in the locus.

[8]Distribution of *dotU* homologs originally noted by Sexton *et al. Infect. Immun.* 2004 Oct;72(10):5983-92.

[9]Bladergroen *et al. Mol. Plant. Microbe Interact*. 2003 Jan;16(1):53-64.

[10]Kinase activity demonstrated in Motley and Lory. *Infect. Immun*. 1999 Oct;67(10):5386-94.

[11]Pallen *et al. Trends Microbiol.* 2002 Dec;10(12):556-63.

Table S2. Summary of clinical sample information.

Patient study	Sample	Sample	Date collected	Patient age	1st positive culture date	Years from 1st positive culture	Strain type
259-3	**1408**	*P. aeruginosa*	5/5/05	13	8/11/99	6	non-mucoid 1
259-3	**1409**	*P. aeruginosa*	5/5/05	13	8/11/99	6	non-mucoid 2
179-5	**0076**	*P. aeruginosa*	9/15/03	8	9/15/03	0	non-mucoid
331-3	**1602**	*P. aeruginosa*	11/15/05	23	before 1993	over 12	non-mucoid
032-5	**0613**	*P. aeruginosa*	12/11/03	4	11/13/03	0.08	mucoid
264-3	**1034**	*P. aeruginosa*	3/23/04	54	before 1993	over 10	mucoid
331-3	**1603**	*P. aeruginosa*	11/15/05	23	before 1993	over 12	mucoid
032-5	**0614**	*P. aeruginosa*	12/11/03	4	11/13/03	0.08	non-mucoid
171-0	**0257**	*P. aeruginosa*	11/07/03	27	before 1993	over 10	mucoid
180-8	"**180-1**" = 1389	*P. aeruginosa*	6/27/05	20	9/19/96	8	-
180-8	"**180-2**" = 1390	*P. aeruginosa*	6/27/05	20	9/19/96	8	-
195-1	"**195-1**" = 0271	*P. aeruginosa*	3/29/05	22	before 1993	over 12	mucoid
195-1	"**195-2**" = 0272	*P. aeruginosa*	3/29/05	22	before 1993	over 12	-
002-6	-	sputum	6/21/05	23	none	-	-
180-8	-	sputum	6/27/05	20	9/19/96	8	-
195-1	-	sputum	3/29/05	22	before 1993	over 12	-
019-1	-	serum	5/27/03	4	-	3-4	-
056-6	-	serum	12/17/03	8	1/17/01	3	-
311-8	-	serum	2/2/04	3	10/13/03	0.3	-
098-0	-	serum	8/7/03	45	-	over 10	-
096-6	-	serum	4/1/04	56	-	over 10	-
251-5	-	serum	2/24/04	36	-	over 10	-

Table S3. Data collection and structure refinement for Hcp1

Data Collection	**Peak**	**Inflection**	**Remote**
Space Group	P6	P6	P6
Cell dimensions (a=b, c in Å)	146.73, 42.22	146.82, 42.24	146.93, 42.24
Wavelength (Å)	0.9794	0.9796	0.9686
Resolution (Å)[a]	50-1.95(2.02)	50-1.95(2.02)	50-1.95(2.02)
I/σI	8.8(1.9)	9.1(1.7)	9.9(2.1)
Completeness %*	98.2 (88.1)	98.0(86.6)	98.6(92.2)
Redundancy	4.4(2.1)	4.4(2.1)	4.5(2.2)

Refinement	
Resolution range (Å)	48-1.95
Number of reflections	35832
R value	0.179
Free R value[b]	0.227
R.m.s. deviation from ideal	
bond length (Å)	0.018
bond angle (°)	1.599
Mean Overall B Value (A^2)	13.24

[a]Highest resolution shell is shown in parenthesis.
[b]5 % of the data were randomly excluded from the refinement to calculate R_{free}.