

SUPPLEMENTARY MATERIAL

Dual control of lysogeny and phage defense by a phosphorylation-based toxin/antitoxin system

Yunxue Guo^{1,2,3#}, Kaihao Tang^{1,2#}, Brandon Sit^{4,5#}, Jiayu Gu^{1,3}, Ran Chen^{1,2}, Jianzhong Lin^{1,3}, Shituan Lin^{1,3}, Xiaoxiao Liu^{1,2}, Weiquan Wang^{1,3}, Xinyu Gao^{1,3}, Zhaolong Nie¹, Tianlang Liu^{1,3}, Matthew K. Waldor^{4,5,6*}, Xiaoxue Wang^{1,2,3*}

*Correspondence to: Xiaoxue Wang, Email: xxwang@scsio.ac.cn; Matthew K. Waldor, E-mail: mwaldor@research.bwh.harvard.edu.

This PDF file includes:

Tables S1, S2, S4, S5, S8 and S9
References

Legends for the Tables S3, S6 and S7 which are uploaded as separate excel files:

Table S3. Published articles with sequencing raw data deposited in the NCBI SRA database. Sequencing reads were mapped to the reference genome of MPAO1 (CP079712). Average depths were calculated of Pf4 and Pf6 genome regions as well as the upstream and downstream 1000 bp regions flanking the prophage genomes. Y indicates yes and N indicates no.

Table S6. Distribution of KKP modules in bacteria. Data were retrieved from the IMG/G database. Gene IDs were indicated.

Table S7. List of phosphorylation sites in PAO1 genome that are modified by KK_{MP} or KKP_{MP} identified by phosphoproteome.

Table S1. List of the Pf4 and Pf6 prophage genes in MPAO1 and their locations in PAO1 genome. The core prophage genes are highlighted in bold letters.

Pf4 in MPAO1 (12416 bp)						Pf6 in MPAO1 (12148 bp)					Nucleotide		Protein	
Locus tag (MPAO1)	Locus tag (PAO1)	Gene name	Start	Stop	Strand	Locus tag (MPAO1)	Gene name	Start	Stop	Strand	Coverage	Identity	Coverage	Identity
MP_4487	PA0729	<i>attR</i>	4717479	4717505	-									
		<i>pfiT</i>	4717628	4717975	-									
MP_4488	PA0728.1	<i>pfiA</i>	4717985	4718236	-		<i>attR</i>	5241438	5241519					
MP_4489	PA0728	<i>intF4</i>	4718450	4719433	-	MP_4975	<i>intF6</i>	5241680	5242681	-	0	0	98%	40.66%
MP_4490	PA0727	<i>repF4</i>	4719433	4720725	-	MP_4976	<i>repF6</i>	5242678	5243889	-	96%	92.30%	93%	95.29%
MP_4491	PA0726.1		4720857	4720958	-	MP_4977		5244102	5244203	-	100%	97.06%	100%	93.94%
MP_4492	PA0726		4720955	4722229	-	MP_4978		5244200	5245474	-	100%	98.27%	100%	97.88%
MP_4493	PA0725	<i>gVI</i>	4722233	4722589	-	MP_4979	<i>gVI</i>	5245478	5245834	-	100%	99.16%	100%	100%
MP_4494	PA0724	<i>gIII</i>	4722594	4723856	-	MP_4980	<i>gIII</i>	5245839	5247098	-	100%	98.10%	100%	98.81%
MP_4495	PA0723	<i>gVIII</i>	4723992	4724240	-	MP_4981	<i>gVIII</i>	5247234	5247482	-	100%	99.20%	100%	100%
MP_4496	PA0722	<i>gIX</i>	4724253	4724504	-	MP_4982	<i>gIX</i>	5247495	5247746	-	100%	100%	100%	100%
MP_4497	PA0721	<i>gVII</i>	4724517	4724609	-	MP_4983	<i>gVII</i>	5247759	5247851	-	100%	97.85%	100%	96.67%
MP_4498	PA0720		4724626	4725060	-	MP_4984		5247868	5248302	-	100%	96.09%	100%	97.92%
MP_4499	PA0719		4725195	4725572	-	MP_4985		5248469	5248813	-	99%	97.38%	79%	98.99%
MP_4500	PA0718		4725576	4725866	-	MP_4986		5248817	5249107	-	100%	96.91%	100%	96.88%
MP_4501	PA0717		4725870	4726082	-	MP_4987		5249111	5249239	-	60%	97.67%	60%	97.62%
MP_4502	PA0716.2	<i>xisF4</i>	4726084	4726299	-	MP_4988	<i>xisF6</i>	5249319	5249549	-	0	0	49%	34.29%
MP_4503	PA0716.1	<i>pf4r</i>	4726418	4726684	+	MP_4989	<i>pf6r</i>	5249717	5249986	+	0	0	100%	50.56%
MP_4504	PA0716		4726973	4728298	-	MP_4990	<i>pfpC</i>	5250121	5251218	+				
MP_4505	PA0715		4728301	4729257	-	MP_4991	<i>pfkB</i>	5251221	5252270	+				
MP_4506	PA0714.1	<i>phrD</i>	4729635	4729707	-	MP_4992	<i>pfkA</i>	5252267	5253340	+				
		<i>attL</i>	4729868	4729894	-		<i>attL</i>	5253510	5253589					

Table S2. The presence of Pf4 and Pf6 loci in the selected *P. aeruginosa* strains were analyzed by BLASTN. The four genomes used in Fig. 2a are shown in blue fonts and two ATCC strains were shown in bold fonts. In addition, the strains with determined sources summarized by C. E., Chandler et al.¹ were also analyzed, and the assembly of these strains were downloaded from NCBI under BioProject accession number PRJNA490649. Y indicates yes and N indicates no.

GenBank/Assembly ID	Strain name	Pf4	Pf6	Source	Date acquired	PubMed ID	Date of publish
MPAO1 sublines							
CP079712	MPAO1	Y	Y	McDermott, University of Washington	07/2020	this study	
CP027857	MPAO1 (C. Manoil)	Y	Y	C. Manoil, University of Washington	2017	33127897	2020 Oct 30
GCA_003957075	MPAO-1 (C. Manoil)	Y	Y	C. Manoil, University of Washington	01/2003	30530517	2019 Feb 11
GCA_003957195	MPAO-1 (C. Manoil)	Y	Y	C. Manoil, University of Washington	08/2008	30530517	2019 Feb 11
GCA_003957175	PAO-1 (B. Iglewski)	Y	Y	E.P. Greenberg, University of Washington	10/2000	30530517	2019 Feb 11
CP050052	PAO1 (B. Iglewski)	Y	Y	E. P. Greenberg, University of Washington	04/2019	unpublished	
GCF_003957105	H103 PAO-1 AK957	Y	Y	R. Hancock, University of British Columbia	02/2000	30530517	2019 Feb 11
GCA_003957205	PAO-1 (B. Holloway)	Y	Y	D. Ohman, Virginia Commonwealth University	05/2001	30530517	2019 Feb 11
GCA_003957095	PAO-1 V	Y	Y	J. Goldberg, University of Virginia	07/2003	30530517	2019 Feb 11
GCA_003957085	PAO-1	Y	Y	H. Nikaido, University of California, Berkeley	08/2003	30530517	2019 Feb 11
GCA_003957115	PAO-1	Y	Y	A. Prince, Columbia University	11/2003	30530517	2019 Feb 11
CP050054/CP050053	PAO1	Y	Y	Y. Liu, South China Agricultural University	04/2018	unpublished	
CP053028	PAO1	Y	Y	S.P. Diggle, Georgia Institute of Technology	01/2014	32341475	2020 Mar 23
not available	PAO1	Y	Y	Scott A. Rice, Nanyang Technological University	not available	34452479	2021 Aug 15
LN871187	PAO1_Orsay1	Y	Y	Pourcel, Institut de Genetique et Microbiologie	not available	unpublished	
CP032126	PAO1161	Y	Y	A. Kawalek, Institute for Biochemistry and Biophysics PAS	11/2017	31906858	2020 Jan 6
NZ_CP017149	PAO-1C (ATCC 15692)	Y	Y	ATCC (deposited by BW Holloway)	not available	unpublished	
CP041008	PAO1 (ATCC BAA-47)	Y	Y	ATCC (deposited by HW Ackermann)	not available	unpublished	
PAO1 sublines							
CP085082	PAO1	Y	N	J.J. Mekalanos, Harvard Medical School	10/2020	this study	
AE004091	PAO1	Y	N	P. Phibbs, University of Georgia	not available	10984043	2000 Aug 31
GCA_003957185	PAO-1	Y	N	J. Burns, University of Washington	02/2000	30530517	2019 Feb 11
CP006832/CP006831	PAO1	Y	N	H.W.D., Yu, Marshall University	not available	24336371	2013 Dec 12
CP006705	PAO1 (PA0581)	Y	N	H.W.D., Yu, Marshall University	not available	24115549	2013 Oct 10
CP053110 to CP053119	PAO1	Y	N	S. Fong, University of Adelaide	12/2017	unpublished	
CP047061 to CP047068	PAO1	Y	N	Balint Csorgo, UCSF	not available	unpublished	
CP032540/CP032541	PAO1	Y	N	Progenesis Technologies, LLC	09/2014	unpublished	
CP034908	PA0750	Y	N	H.P. Schweizer, Colorado State University	2005	17005832	2006 Oct

Table S4. RNA-seq reads of each Pf4 and Pf6 genes in the biofilm cells and in the planktonic cells of MPAO1 strain. Three replicates were used and shown. NA indicates not available. FC, CPM and FDR indicate fold change, counts per million and false discovery rate, respectively.

Locus tag (MPAO1)	Gene product	Planktonic cells			Biofilm cells			LogFC	LogCPM	FDR
		# 1	# 2	# 3	# 1	# 2	# 3			
Pf4 prophage										
MP_4487	PfiT	59.0	69.9	45.6	583.1	272.6	468.9	3.0	6.4	5.29E-36
MP_4488	PfiA	60.2	47.0	63.6	539.2	302.2	369.8	2.9	5.9	1.55E-24
MP_4489	IntF4	1.7	4.0	2.3	459.7	103.6	155.5	6.5	6.8	2.72E-44
MP_4490	RepF4	2.9	3.8	3.6	892.8	196.0	316.5	7.1	8.2	6.83E-54
MP_4491	Hypothetical	0.0	0.0	0.0	41.5	1.8	9.3	6.3	0.1	0.00088
MP_4492	Hypothetical	1.0	0.6	1.0	576.3	122.4	177.4	8.3	7.5	8.38E-53
MP_4493	pVI	0.0	0.9	0.0	23.7	3.0	0.5	4.7	0.8	0.001108
MP_4494	pIII	0.3	1.2	1.3	1196.3	249.6	426.0	9.4	8.5	4.14E-62
MP_4495	pVIII	1.3	4.3	6.4	11773.3	1518.9	1889.6	10.2	9.2	2.20E-35
MP_4496	pIX	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA	NA
MP_4497	pVII	0.0	0.0	0.0	123.4	15.5	34.5	7.9	1.4	1.12E-08
MP_4498	Hypothetical	8.3	23.8	9.2	20618.8	4612.1	8445.6	9.7	11.2	5.79E-77
MP_4499	Hypothetical	8.6	9.7	0.9	9635.9	2351.2	4761.3	9.8	10.0	1.07E-71
MP_4500	Hypothetical	3.4	4.7	8.8	5423.0	2356.9	3570.8	9.5	9.1	8.37-126
MP_4501	Hypothetical	0.8	0.7	3.0	1681.4	588.2	744.9	9.4	6.7	7.87E-83
MP_4502	XisF4	2.3	1.4	2.2	5079.5	950.1	1656.8	10.3	8.0	1.58E-62
MP_4503	Pf4r	24.4	23.9	18.6	48.3	58.0	110.9	1.9	3.7	1.36E-06
MP_4504	Hypothetical	48.2	38.2	32.7	39.9	68.0	94.3	0.99	6.2	0.01418
MP_4505	Hypothetical	48.4	22.9	23.4	33.9	60.2	91.3	1.2	5.6	0.01255
MP_4506	PhrD	464.6	878.3	875.3	391.6	254.0	434.2	-0.92	5.3	0.00379
Pf6 prophage										
MP_4975	IntF6	4.9	2.3	3.8	61.9	93.2	142.0	4.9	5.8	9.62E-27
MP_4976	RepF6	3.2	2.5	2.1	164.9	237.5	393.0	6.9	7.5	4.69E-49
MP_4977	Hypothetical	0.0	0.0	0.0	63.2	88.2	160.9	9.1	2.5	1.01E-18
MP_4978	Hypothetical	0.1	0.6	0.5	70.1	135.1	200.5	8.6	6.6	1.07E-44
MP_4979	pVI	0.0	0.0	0.0	10.7	9.1	9.5	7.5	1.0	2.15E-09

MP_4980	pIII	1.0	0.5	1.0	189.8	335.9	525.1	8.9	7.9	1.92E-52
MP_4981	pVIII	0.0	1.2	2.6	3837.9	2455.9	2303.2	11.2	8.5	8.44E-126
MP_4982	pIX	0.0	0.0	0.0	0.0	0.0	0.0	NA	NA	NA
MP_4983	pVII	0.0	0.0	0.0	10.8	7.7	24.3	6.0	-0.1	0.000402
MP_4984	Hypothetical	4.1	6.3	4.4	4002.7	4457.3	6895.2	10.2	10.2	1.91E-110
MP_4985	Hypothetical	3.8	4.4	0.9	4505.9	4766.5	8160.2	11.1	10.1	4.38E-108
MP_4986	Hypothetical	0.6	1.0	2.2	2020.1	2039.6	3080.0	11.0	8.5	8.22E-108
MP_4987	Hypothetical	2.5	0.0	2.5	1373.3	1618.7	1675.9	10.0	6.8	4.59E-81
MP_4988	XisF6	3.5	4.6	1.4	4576.2	6590.4	10843.9	11.4	9.9	1.21E-85
MP_4989	Pf6r	186.0	146.3	99.1	1427.1	462.0	666.6	2.6	7.0	8.03E-17
MP_4990	PfpC	93.1	67.4	46.4	468.6	209.2	306.5	2.3	7.8	1.02E-16
MP_4991	PfkB	162.3	109.5	76.3	2198.4	953.6	1415.6	3.8	9.7	3.64E-34
MP_4992	PfkA	731.0	674.1	431.5	933.8	319.8	466.5	-0.02	9.3	0.97

Table S5. List of proteins that are modified by PfkA kinase identified by phosphoproteome. The fold changes indicated the phosphorylation levels of proteins controlled by PfkA via pHERD20T-*pfkA* overproduction versus those in cells harboring pHERD20T vector. Two replicates were used, and those proteins with fold changes > 2.5 and coefficient of variation (CV) < 0.1) were selected.

Locus tag (MPAO1)	Locus tag (PAO1)	Protein	Position	Fold change	CV value	Description
MP_2627	PA2480		391T	4.554	0.006	Signal transduction histidine kinase
MP_4036	PA1155	NrdB	408T	3.968	0.008	Ribonucleotide-diphosphate reductase subunit beta
MP_618	PA0577	DnaG	527T	3.816	0.088	DNA primase
MP_1549	PA3481	ApbC	353S	3.777	0.022	ATPases involved in chromosome partitioning
MP_3583	PA1585	SucA	62T	3.616	0.072	Dehydrogenase (E1) component related enzymes
MP_82	PA0074	PpkA	270T	3.261	0.087	Ser/Thr kinase
MP_723	PA4274	RplK	71T	3.101	0.046	Ribosomal protein L11
MP_5588	PA5239	Rho	84S	3.010	0.060	Transcription termination factor
MP_2467	PA2629	PurB	296S	2.975	0.088	Adenylosuccinate lyase
MP_2427	PA2667	MvaU	65T/67S	2.892	0.019	H-NS family protein
MP_5084	PA4759	DapB	207T	2.640	0.069	Dihydrodipicolinate reductase
MP_4426	PA0787		79S	2.624	0.089	Predicted ATPase
MP_5631	PA5279		49S	2.609	0.085	Uncharacterized protein conserved in bacteria
MP_5697	PA5343		246S	2.604	0.026	Nucleoside-diphosphate-sugar epimerases
MP_5797	PA5435	PycB	462T	2.517	0.022	Pyruvate/oxaloacetate carboxyltransferase
MP_1500	PA3529		198S	0.229	0.049	Peroxidase
MP_1153	PA3861	RhlB	38S	0.129	0.044	ATP-dependent RNA helicase RhlB
MP_180	PA0170		68T	0.017	0.083	DUF1987 domain-containing protein

Table S8. Bacterial strains and plasmids used in this study. R indicates resistance, Gm indicates gentamycin, Amp indicates ampicillin, MP indicates MPAO1 strain. KKP indicates the *pfkA-pfkB-pfpC* cascade.

	Description	Source
Strains		
PAO1	wild-type	2
MPAO1	wild-type	3
MP-ΔP _{f4}	whole P _{f4} prophage removed from MPAO1 host chromosome	3
MP-ΔP _{f6} core	the core genes from MP_4976 to MP_4987 removed from MPAO1 host chromosome	this study
MP-ΔP _{f4} ΔP _{f6} core	whole P _{f4} prophage removed from MP-ΔP _{f6} core host chromosome	this study
pVIII::GFP	<i>gVIII</i> gene encoding P _{f4} major coat protein pVIII in-frame fused with <i>gfp</i> gene in PAO1 host chromosome	this study
MP-pVIII::GFP	<i>gVIII</i> gene encoding P _{f4} major coat protein pVIII in-frame fused with <i>gfp</i> gene in MPAO1 host chromosome	this study
MP-Δ <i>mvaU</i> Δ <i>mvaT</i>	<i>mvaT</i> and <i>mvaU</i> double deletion mutant derived from MPAO1	3
PAO1:: <i>pilC</i> _{MP}	<i>pilC</i> gene from MPAO1 was integrated into Δ <i>pilC</i> host chromosome	this study
PAO1:: <i>pilC</i> _{MP} ::KKP _{MP}	the KKP _{MP} cascades were integrated into PAO1:: <i>pilC</i> _{MP} host chromosome	this study
PAO1::KKP _{MP}	the KKP _{MP} cascades were integrated into PAO1 host chromosome	this study
PAO1:: <i>pilC</i> _{MP} ::K ^{GFI} KP _{MP}	the mutant KKP _{MP} cascade (the 307 th -309 th aa codon GGATTCATT in <i>pfkA</i> was mutated to GACTATGCA) were integrated into PAO1:: <i>pilC</i> _{MP} host chromosome	this study
WM3064	<i>thrB1004 pro thi rpsL hsdS lacZ</i> ΔM15 RP4-1360) Δ(<i>araBAD</i>)567 Δ <i>dapA</i> 1341::[<i>erm pir</i> (wt)]	W. Metcalf, UIUC
MG655	K-12 <i>F⁻ lambda⁻ ilvG⁻ rfb-50 rph-1</i>	lab stored
Plasmids		
pEX18Ap	Ap ^R , <i>oriT</i> ⁺ , <i>sacB</i> ⁺ , gene replacement vector	4
pEX18Gm	Gm ^R , <i>oriT</i> ⁺ , <i>sacB</i> ⁺ , gene replacement vector	4
pFLP2	Ap ^R , Flp recombinase-expressing plasmid	4
pPS856	Ap ^R , Gm ^R ; for amplifying gentamycin resistance cassette	4
pEX18Gm-ΔP _{f6} core-up-down	Gm ^R , Ap ^R , for deleting P _{f6} core region	this study
pEX18Gm-up-pVIII::GFP-down	Gm ^R , Ap ^R , for P _{f4} <i>gVIII</i> in-frame fused with <i>gfp</i> gene in MPAO1 host chromosome	this study
pEX18Gm- <i>pilC</i> -up-down	Gm ^R , Ap ^R , for deleting <i>pilC</i> in PAO1 host chromosome	this study
pEX18Gm-up- <i>pilC</i> _{MP} -down	Gm ^R , Ap ^R , for integration of <i>pilC</i> _{MP} gene from MPAO1 into Δ <i>pilC</i> host chromosome	this study
pEX18Gm-up-Gm-KKP-down	Gm ^R , Ap ^R , for integration of KKP _{MP} cascade from MPAO1 into PAO1:: <i>pilC</i> _{MP} host chromosome	this study
pEX18Gm-up-Gm-K ^{GFI} KP-down	Gm ^R , Ap ^R , for integration of K ^{GFI} KP _{MP} cascade mutant into PAO1:: <i>pilC</i> _{MP} host chromosome	this study
pHERD20T	Ap ^R , expression vector with araC-P _{BAD} promoter	5
pHERD20T- <i>mvaU</i>	Ap ^R , <i>mvaU</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>mvaU</i> ^{S2A}	Ap ^R , the second aa codon TCC in <i>mvaU</i> was mutated to GCC in pHERD20T- <i>mvaU</i>	this study

pHERD20T- <i>mvaU</i> ^{S2D}	Ap ^R , the second aa codon TCC in <i>mvaU</i> was mutated to GAC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{S26A}	Ap ^R , the 26 th aa codon AGC in <i>mvaU</i> was mutated to GCC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{S26D}	Ap ^R , the 26 th aa codon AGC in <i>mvaU</i> was mutated to GAC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{T50A}	Ap ^R , the 50 th aa codon ACC in <i>mvaU</i> was mutated to GCC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{T50D}	Ap ^R , the 50 th aa codon ACC in <i>mvaU</i> was mutated to GAC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{T65A}	Ap ^R , the 65 th aa codon ACC in <i>mvaU</i> was mutated to GCC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{T65D}	Ap ^R , the 65 th aa codon ACC in <i>mvaU</i> was mutated to GAC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{S67A}	Ap ^R , the 67 th aa codon AGC in <i>mvaU</i> was mutated to GCC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{S67D}	Ap ^R , the 67 th aa codon AGC in <i>mvaU</i> was mutated to GAC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{S108A}	Ap ^R , the 108 th aa codon TCC in <i>mvaU</i> was mutated to GCC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{S108D}	Ap ^R , the 108 th aa codon TCC in <i>mvaU</i> was mutated to GAC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{S113A}	Ap ^R , the 113 th aa codon TCC in <i>mvaU</i> was mutated to GCC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> ^{S113D}	Ap ^R , the 113 th aa codon TCC in <i>mvaU</i> was mutated to GAC in pHERD20T- <i>mvaU</i>	this study
pHERD20T- <i>mvaU</i> -His- <i>pfkA</i>	Ap ^R , N terminal His tagged <i>mvaU</i> was coexpressed with <i>pfkA</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>mvaU</i> -His- <i>pfkB</i>	Ap ^R , N terminal His tagged <i>mvaU</i> was coexpressed with <i>pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>mvaU</i> -His- <i>pfkA-pfkB</i>	Ap ^R , N terminal His tagged <i>mvaU</i> was coexpressed with <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>mvaU</i> -His-KKP _{MP}	Ap ^R , N terminal His tagged <i>mvaU</i> was coexpressed with KKP _{MP} in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i>	Ap ^R , <i>pfkA</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{CSD}	Ap ^R , mutant <i>pfkA</i> lacking CSD domain in pHERD20T NcoI/HindIII	this study
pHERD20T-CSD	Ap ^R , CSD domain of <i>pfkA</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkB</i>	Ap ^R , <i>pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkB</i> ^{FHA}	Ap ^R , mutant <i>pfkB</i> lacking FHA domain in pHERD20T NcoI/HindIII	this study
pHERD20T-FHA	Ap ^R , FHA domain of <i>pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfpC</i>	Ap ^R , <i>pfpc</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA-pfkB</i>	Ap ^R , <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{CSD} - <i>pfkB</i>	Ap ^R , mutant <i>pfkA-pfkB</i> without CSD domain in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA-pfkB</i> ^{FHA}	Ap ^R , mutant <i>pfkA-pfkB</i> without FHA domain in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{CSD} - <i>pfkB</i> ^{FHA}	Ap ^R , mutant <i>pfkA-pfkB</i> without both CSD and FHA domains in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{GGM} - <i>pfkB</i>	Ap ^R , mutant GGM motif in PfkA in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{HRD} - <i>pfkB</i>	Ap ^R , mutant HRD motif in PfkA in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{DFG} - <i>pfkB</i>	Ap ^R , mutant DFG motif in PfkA in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{GFI} - <i>pfkB</i>	Ap ^R , mutant GFI motif in PfkA in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA-pfkB</i> ^{GGM}	Ap ^R , mutant GGM motif in PfkB in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study

pHERD20T- <i>pfkA-pfkB</i> ^{HRD}	Ap ^R , mutant HRD motif in PfkB in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA-pfkB</i> ^{DFG}	Ap ^R , mutant DFG motif in PfkB in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{GGM} - <i>pfkB</i> ^{GGM}	Ap ^R , mutant GGM motifs in both PfkA and PfkB in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{HRD} - <i>pfkB</i> ^{HRD}	Ap ^R , mutant HRD motifs in both PfkA and PfkB in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{DFG} - <i>pfkB</i> ^{DFG}	Ap ^R , mutant DFG motifs in both PfkA and PfkB in <i>pfkA-pfkB</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{GGM+HRD+DFG} - <i>pfkB</i>	Ap ^R , the GGM, HRD and DFG motif in PfkA were mutated as PAO1::MP- <i>pilC</i> ::K ^{GGM+HRD+DFG} K ^{GGM+HRD+DFG} P in <i>pfkA-pfkB</i> , and the generated <i>pfkA</i> ^{GGM+HRD+DFG} - <i>pfkB</i> was ligated to pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA-pfkB</i> ^{GGM+HRD+DFG}	Ap ^R , the GGM, HRD and DFG motif in PfkB were mutated as PAO1::MP- <i>pilC</i> ::K ^{GGM+HRD+DFG} K ^{GGM+HRD+DFG} P in <i>pfkA-pfkB</i> , and the generated <i>pfkA</i> ^{GGM+HRD+DFG} - <i>pfkB</i> was ligated to pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{GGM+HRD+DFG} - <i>pfkB</i> ^{GGM+HRD+DFG}	Ap ^R , the GGM, HRD and DFG motif in both PfkA and PfkB were mutated as PAO1::MP- <i>pilC</i> ::K ^{GGM+HRD+DFG} K ^{GGM+HRD+DFG} P in <i>pfkA-pfkB</i> , and the generated <i>pfkA</i> ^{GGM+HRD+DFG} - <i>pfkB</i> ^{GGM+HRD+DFG} was ligated to pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA-pfpC</i>	Ap ^R , <i>pfkA-pfpC</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkB-pfpC</i>	Ap ^R , <i>pfkB-pfpC</i> in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA-pfkB-pfpC</i>	Ap ^R , KKP _{MP} (<i>pfkA-pfkB-pfpC</i>) in pHERD20T NcoI/HindIII	this study
pHERD20T- <i>pfkA</i> ^{GGM} - <i>pfkB</i>	Ap ^R , the 23 th -25 th aa codon GGAGGCATG in <i>pfkA</i> was mutated to GCAGCGTGT in pHERD20T- <i>pfkA-pfkB</i>	this study
pHERD20T- <i>pfkA</i> ^{HRD} - <i>pfkB</i>	Ap ^R , the 131 th -133 th aa codon CATCGCGAC in <i>pfkA</i> was mutated to TTCGAAAAT in pHERD20T- <i>pfkA-pfkB</i>	this study
pHERD20T- <i>pfkA</i> ^{DFG} - <i>pfkB</i>	Ap ^R , the 154 th -157 th aa codon GACTTCGGT in <i>pfkA</i> was mutated to AATTATGCA in pHERD20T- <i>pfkA-pfkB</i>	this study
pHERD20T- <i>pfkA-pfkB</i> ^{GGM}	Ap ^R , the 16 th -18 th aa codon GGCGGCATG in <i>pfkB</i> was mutated to GCAGCGTGT in pHERD20T- <i>pfkA-pfkB</i>	this study
pHERD20T- <i>pfkA-pfkB</i> ^{HRD}	Ap ^R , the 121 th -123 th aa codon CATAGAGAT in <i>pfkB</i> was mutated to TTCGAAAAT in pHERD20T- <i>pfkA-pfkB</i>	this study
pHERD20T- <i>pfkA-pfkB</i> ^{DFG}	Ap ^R , the 141 th -143 th aa codon GACTTTGGC in <i>pfkB</i> was mutated to AATTATGCA in pHERD20T- <i>pfkA-pfkB</i>	this study
pHERD20T-2208	Ap ^R , 2208 in pHERD20T NcoI/HindIII	this study
pHERD20T-2209	Ap ^R , 2209 in pHERD20T NcoI/HindIII	this study
pHERD20T-2210	Ap ^R , 2210 in pHERD20T NcoI/HindIII	this study
pHERD20T-2208-2209	Ap ^R , 2208-2209 in pHERD20T NcoI/HindIII	this study
pHERD20T-2208-2210	Ap ^R , 2208-2210 in pHERD20T NcoI/HindIII	this study
pHERD20T-2209-2210	Ap ^R , 2209-2210 in pHERD20T NcoI/HindIII	this study
pHERD20T-2208-2209-2210	Ap ^R , 2208-2209-2210 (KKP _{SW} in marine <i>Shewanella</i> W3-18-1) in pHERD20T NcoI/HindIII	this study
pHERD20T-K ^{GFA} -KP _{SW}	Ap ^R , 2208 ^{GFA} -2209-2210 (K ^{GFA} KP _{SW} with GFA mutation in marine <i>Shewanella</i> W3-18-1) in pHERD20T NcoI/HindIII	this study
pHERD20T-K ^{HRD+DFG} -K ^{HRD+DFG} P _{SW}	Ap ^R , 2208-2209 ^{HRD+DFG} -2210 ^{HRD+DFG} (K ^{HRD+DFG} K ^{HRD+DFG} P _{SW} with GFA mutation in marine <i>Shewanella</i> W3-18-1) in pHERD20T NcoI/HindIII	this study
pHERD20T-K ^{GFI} -KP _{EC039}	Ap ^R , GFI mutation in <i>E. coli</i> 15EC039 KKP _{EC039} (K ^{GFI} KP _{EC039}) in pHERD20T NcoI/HindIII	this study

Table. S9 Oligonucleotides used for gene knockout and DNA sequencing. F indicates forward primer and R indicates reverse primer. The red letters indicate the mutation of nucleotides.

Primer name	Sequence (5'-3')	Purpose
Primers used for construction of MP-ΔPf6core and MP-ΔPf4ΔPf6core		
Pf6core-up-F	acgacggccagtgccaagcttTCTAAGTTCACATTCATGCACTCTTTC	Construction of pEX18Gm-ΔPf6core-up-down
Pf6core-up-R	tgagcgggtctcaTTAGTCACCGTCGTAATCCCCC	
Pf6core-down-F	tgactaaTGAGACCCGCTCAATGGACA	
Pf6core-down-R	ggtacccggggatcctctagaGAGTGGTGATAGCGAAACTCGAT	
Pf6core-conf-SF	TGAATCATCGCTACGCTCCTC	Verification of Pf6core knockout in MP-ΔPf6core and MP-ΔPf4ΔPf6core
Pf6core-conf-SR	ACGGCTGCTTGGTTGGTCT	
Pf6core-conf-LF	CATGGCAGACTCTATCAGCCA	
Pf6core-conf-LR	ATCGCATATCCCTTGCGCAGATA	
Primers used for construction of pVIII::GFP and MP-pVIII::GFP		
pVIII-gfp-up-F	gccagtgccaagcttgcattgcAGGACAAACAAGACAAGACCCCCG	Construction of pEX18Gm-pVIII::GFP-up-down
pVIII-gfp-up-R	TTCTCCTTTACTCGCCTTGCGCAACATGCT	
pVIII-gfp-F2	GCAAGGCGAGTAAAGGAGAAGAAGCTTTTCACTGGA	
pVIII-gfp-R2	CCAGAGCACCCGTTATTTGTATAGTTCATCCATGCCATG	
pVIII-gfp-down-F	ACAAATAACGGGTGCTCTGGTCGGTG	Verification of strains pVIII::GFP and MP-pVIII::GFP
pVIII-gfp-down-R	tatgaccatgattacgaattcCGGTGCCCTTGAGGATGTAA	
pVIII-gfp-conf-SF	GCGAATACAACATCGAGCCG	
pVIII-gfp-conf-SR	GGTCTGGGAGAAGGTGTAGGAAT	
pVIII-gfp-conf-LF	ATGAACATGTTTGCAACCCAA	
pVIII-gfp-conf-LR	TCAGTCCTTCAGCAGAATGAG	
Primers used for construction of ΔpilC and PAO1::pilCMP		
pilC-up-F	acgacggccagtgccaagcttTCTGCTCGTCTCAAGGTAAT	Construction of pEX18Gm-up-pilC-down, and pEX18Gm-up-pilCMP-down
pilC-up-R	ATGGCTGGCCAGGTAGTCGAGGAGGGGCATGGATTAATCCTTGGTCACGCGGTTGACTT	
pilC-down-F	AAGTCAACCGCGTGACCAAGGATTAATCCATGCCCTCCTCGACTACCTGGCCAGCCAT	
pilC-down-R	tatgaccatgattacgaattcAGTTGGTGATCGGCATCGAT	
pilC-conf-SF	GAGCAAGCCCGCAAAGAAG	Verification of strains ΔpilC, and PAO1::pilCMP
pilC-conf-SR	CCAGTTGCGCTCCATCATCT	
pilC-conf-LF	AAGATGCTGCTGGATGCCAT	
pilC-conf-LR	GATCAGCGGGTGCAACAATT	

Primers used for construction of PAO1::*pilC*_{MP}::KKP_{MP} and PAO1::*pilC*_{MP}::K^{GFI}KP_{MP}

KKP _{MP} -F1	acgacggccagtgccaagcttTAGTTGATTACCACTGAGGAGGTGG
KKP _{MP} -R1	CGACCCAAGTACCGCCACCTAAATGGCACTCTAATAAAATAAAATTAAATTTATCGCTTC
KKP _{MP} -F2	GAAGCGATAAAATTTAATTTTATTTATTAGAGTGCCATTTAGGTGGCGGTACTTGGGTCG
KKP _{MP} -R2	AGCACATCCTGTTATGATCCTTGATGAGTAGAGTTCCTATACTTTCTAGAGAATAGGAA
KKP _{MP} -F3	TTCTATTCTCTAGAAAGTATAGGAACTCTACTCATCAAGGATCATAACAGGATGTGCT
KKP _{MP} -R3	TGTCCCAGGTTCAAGTCCCGGTGTAGCCACCATGAGAATCAATACAGAGCAGAGCTAGG
KKP _{MP} -F4	CCTAGCTCTGCTCTGTATTGATTCTCATGGTGGCTACACCGGGACTTGAACCTGGGACA
KKP _{MP} -R4	tatgaccatgattacgaattcGGGCAAGCCGGCGCGTTC
KK ^{GFI} P _{MP} -R3	GAAAATTTCTTTCCCCATCAAGGATGACTAGGAATGAGCACAATACCAGACCGATATGA
KK ^{GFI} P _{MP} -F4	TCATATCGGTCTGGTATTGTGCTCATTCCTAGTCATCCTTGATGGGGAAAGAAATTTTC

Amplification of the 697 bp downstream of *pfkA*

Amplification of Gm resistance gene with its 303 bp promoter

Amplification of KKP cascades and upstream 404 bp promoter the start codon of *pf6r* was mutated to TGA)

Amplification of the 836 bp from Pf6 *attR* to upstream

Amplification of *pfkA*^{GFI}-*pfkB* from pHERD20T-*pfkA*^{GFI}-*pfkB*

Amplification of *ppfC* and the 836 bp from Pf6 *attR* to upstream with KKP_{MP}-R4, the template is PAO1::*pilC*_{MP}::KKP_{MP} genomic DNA

Primers used for construction of pHERD20T-*mvaU* and related mutants

pHERD20T- <i>mvaU</i> -F	aagaaggagatatacataccATGTCCAAACTTGCCGAGTTC
pHERD20T- <i>mvaU</i> -R	cgcggccagtgccaagcttTTAGCGTTGCAGCCAGGATTC
pHERD20T- <i>mvaU</i> ^{S2A} -F	aagaaggagatatacataccATGGCCAAACTTGCCGAGTTC
pHERD20T- <i>mvaU</i> ^{S2D} -F	aagaaggagatatacataccATGGACAAACTTGCCGAGTTC
pHERD20T- <i>mvaU</i> ^{S26A} -F	GCTGAAA ^{GCC} GACAGCAGCCTGAAGCAGGAACTGGAAT
pHERD20T- <i>mvaU</i> ^{S26A} -R	GCTGCTGTC ^{GGC} TTTCAGCTTTTCCAGCAGGGCCAGTT
pHERD20T- <i>mvaU</i> ^{S26D} -F	AAGCTGAAA ^{GAC} GACAGCAGCCTGAAGCAGGAACTGGAAT
pHERD20T- <i>mvaU</i> ^{S26D} -R	CAGGCTGCTGTC ^{GTC} TTTCAGCTTTTCCAGCAGGGCCAGT
pHERD20T- <i>mvaU</i> ^{T50A} -F	TACGGCATG ^{GCC} CTGCACAACATCATCGCCATCCTCGACC
pHERD20T- <i>mvaU</i> ^{T50A} -R	GTTGTGCAG ^{GGC} CATGCCGTACTTGTCCATCAACGCCTGC
pHERD20T- <i>mvaU</i> ^{T50D} -F	TACGGCATG ^{GAC} CTGCACAACATCATCGCCATCCTCGACC
pHERD20T- <i>mvaU</i> ^{T50D} -R	GTTGTGCAG ^{GTC} CATGCCGTACTTGTCCATCAACGCCTGC
pHERD20T- <i>mvaU</i> ^{T65A} -F	AAGGCTCCGGTC ^{GCC} GTCAGCGCCGCTCCGCAGCGCCGTGCCCCG
pHERD20T- <i>mvaU</i> ^{T65A} -R	GGCGCTGAC ^{GGC} GACCGGAGCCTTGGGGTCGAGGATGGCGATGAT
pHERD20T- <i>mvaU</i> ^{T65D} -F	GCTCCGGTC ^{GAC} GTCAGCGCCGCTCCGCAGCGCCGTGCC

Construction of pHERD20T-*mvaU* and related single site mutations in MvaU

pHERD20T- <i>mvaU</i> ^{T65D} -R	GCTGAC GT CGACCGGAGCCTTGGGGTCGAGGATGGCGAT
pHERD20T- <i>mvaU</i> ^{S67A} -F	AAGGCTCCGGTCACCGTC GCC GCCGCTCCGCAGCGCCGTGCCCCG
pHERD20T- <i>mvaU</i> ^{S67A} -R	GG CGGC GACGGTGACCGGAGCCTTGGGGTCGAGGATGGCGATGAT
pHERD20T- <i>mvaU</i> ^{S67D} -F	GCTCCGGTCACCGTC GAC GCCGCTCCGCAGCGCCGTGCC
pHERD20T- <i>mvaU</i> ^{S67D} -R	GGAGCGGC GT CGACGGTGACCGGAGCCTTGGGGTCGAGGATGGCGAT
pHERD20T- <i>mvaU</i> ^{S108A} -R	cgacggccagtccaagcttTTAGCGTTGCAGCCAGGATTTCGACGGTTTCGGAACCG GC CTGTTCTTTC
pHERD20T- <i>mvaU</i> ^{S108D} -R	cgacggccagtccaagcttTTAGCGTTGCAGCCAGGATTTCGACGGTTTCGGAACCG GT CTGTTCTTTC
pHERD20T- <i>mvaU</i> ^{S113A} -R	cgacggccagtccaagcttTTAGCGTTGCAGCCA GG CTTCGACGGTTTC
pHERD20T- <i>mvaU</i> ^{S113D} -R	cgacggccagtccaagcttTTAGCGTTGCAGCCA GT CTTCGACGGTTTC

Primers used for cloning KKP_{MP} components and domains of PfkA and PfkB

pHERD20T- <i>pfkA</i> -F	aggagatatacatccatgACGACTTCGAGAATCGGAAAGAC
pHERD20T- <i>pfkA</i> -R	acgacggccagtccaagcttCTACTCATCAAGGATCATAACAGGATG
pHERD20T- <i>pfkB</i> -F	aggagatatacatccatgAGCACAATACCAGACCGATATGAATT
pHERD20T- <i>pfkB</i> -R	acgacggccagtccaagcttTCATAAGTTTATCTCCGGATGAGATAG
pHERD20T- <i>pfpC</i> -F	aggagatatacatccatgAATGTGAACCTTAGAGAACGATATTACATTTTC
pHERD20T- <i>pfpC</i> -R	acgacggccagtccaagcttCTAGTCATCCTTGATGGGGAAAGA
<i>pfkA</i> ^{CSD} -R	acgacggccagtccaagcttTCAACATAAAAGTTCACATTTTTTTTACC
<i>pfkB</i> ^{FHA} -R1	TTAGCGCTTGATGTCTGTTTTCTAACTATATGTGCTTCTCAAGTGTCTCTTTGATTGAC
<i>pfkB</i> ^{FHA} -F2	GTCAATCAAAGAGACACTTGAGAAGCACATAT AG TTAGAAAACAGACATCAAGCGCTAA
pHERD20T- <i>pfkA</i> ^{CSD} -R	acgacggccagtccaagctt TC ATATGTGCTTCTCAAGTGTCTCTTT
pHERD20T- <i>CSD</i> -R	aggagatatacatacc ATG TTAGAAAACAGACATCAAGCGC
pHERD20T- <i>pfkB</i> ^{FHA} -R	acgacggccagtccaagctt CTA ACATAAAAGTTCACATTTTTTTTACCA
pHERD20T- <i>FHA</i> -R	aggagatatacatacc ATG TATGCAAAAAACCCAAGATATATTG

Primers used for construction of conserved domains in pHERD20T-*pfkA*-*pfkB*

pHERD20T- <i>pfkA</i> ^{GM} - <i>pfkB</i> -F	ATCTCGATATTTAATCGAGGACTTAATCGGCGAA GCAGCGTG TCAATATGTTTACCG
pHERD20T- <i>pfkA</i> ^{GM} - <i>pfkB</i> -R	CGGTAAACATATTGACACGCTGCTTCGCCGATTAAAGTCCTCGATTAAATATCGAGAT
pHERD20T- <i>pfkA</i> ^{HRD} - <i>pfkB</i> -F	TGCCGGAGTAGTT TTTCGAAAAT CTGAAACCAAC
pHERD20T- <i>pfkA</i> ^{HRD} - <i>pfkB</i> -R	GTTGGTTTCAGATTTTCGAAAACACTACTCCGGCA
pHERD20T- <i>pfkA</i> ^{DFG} - <i>pfkB</i> -F	GTAAAAAATTACT AATTATGCA ATTGCAAAAATG
pHERD20T- <i>pfkA</i> ^{DFG} - <i>pfkB</i> -R	CATTTTTGCAATTGCATAATTAGTAATTTTAAAC

Construction of pHERD20T-based constructions for expression of MPAO1 KKP components

Construction of pHERD20T-*pfkA*^{CSD}-*pfkB*, pHERD20T-*pfkA*-*pfkB*^{FHA} and pHERD20T-*pfkA*^{CSD}-*pfkB*^{FHA} together with primers pHERD20T-*pfkB*-F and pHERD20T-*pfkA*-R
Construction of pHERD20T-*pfkA*^{CSD}, pHERD20T-*pfkA*^{CSD}, pHERD20T-*pfkB*^{FHA} and pHERD20T-*FHA* together with primers pHERD20T-*pfkB*-F/R and pHERD20T-*pfkA*-F/R

F primers/HERD20T-*pfkA*-R and pHERD20T-*pfkB*-F/R primers were used to amplify the second and first fragments separately, the two fragments were fused using pHERD20T-*pfkB*-F and pHERD20T-*pfkA*-R and ligated into pHERD20T plasmid.

pHERD20T- <i>pfkA</i> - <i>pfkB</i> ^{GGM} -F	TTGAT GCAGCGTTA GGTACAATCTTCGTCTGC
pHERD20T- <i>pfkA</i> - <i>pfkB</i> ^{GGM} -R	GCAGACGAAGATTGTACCTAACGCTGCATCAA
pHERD20T- <i>pfkA</i> - <i>pfkB</i> ^{HRD} -F	GTAAACATAATT TTCGAAAAT ATCAAACCAAACAAC
pHERD20T- <i>pfkA</i> - <i>pfkB</i> ^{HRD} -R	GTTGTTTGGTTTGATATTTTCGAAAATTATGTTTAC
pHERD20T- <i>pfkA</i> - <i>pfkB</i> ^{DFG} -F	ATCATTAATAATTT AATTATGC ATTAGCAAGACACAC
pHERD20T- <i>pfkA</i> - <i>pfkB</i> ^{DFG} -R	GTGTGTCTTGCTAATGCATAATTAAATATTTTAATGAT
<i>pfkA</i> - <i>pfkB</i> -linker-F	TTACGTTTCGACCTATCTCATCCGGAGATAAACTTATGACGACTTCGAGAATCGGAAAGACTATA
<i>pfkA</i> - <i>pfkB</i> -linker-R	TATAGTCTTCCGATTCTCGAAGTCGTCATAAGTTTATCTCCGGATGAGATAGGTCGAACGTAA

Construction the double GGM, HDR, DFG mutations, the *pfkB* and *pfkA* single mutants were amplified using the above PCR products as templates, and fused with the primers and ligated into pHERD20T plasmid.

<i>pfkB</i> -in-R1	TAAGTCATTGCCGTAGATATATTCTTGTACGATGCCAAGCCCGCGTTCCG
<i>pfkB</i> -in-F1	CGAACGCGGGCTTGGCATCGTACAAGAATATATCTACGGCAATGACTTA
<i>pfkB</i> -in-R2	AATATTTTAATGATGCTTTCATGGTCTAGCATCATGTTGTTTGGTTTGA
<i>pfkB</i> -in-F2	TCAAACCAAACAACATGATGCTAGACCATGAAAGCATCATTAATAATTT
<i>pfkA</i> -in-R1	GGCTACGTTATGATGATTGACTTTGGCTGCTACAATGGCACTACGCCG
<i>pfkA</i> -in-F1	CGGCGTAGTGCCATTGTAGCAGCCAAAGTCAATCATCATAACGTAGCC
<i>pfkA</i> -in-R2	AACTCATTTAGTGAATATCCGCCAGAGATCATAACGTTTGTGGTTTCA
<i>pfkA</i> -in-F2	TGAAACCAACAAACGTTATGATCTCTGGCGGATATTCATAAATGAGTT

Construction of pHERD20T-*pfkA*^{GGM+HRD+DFG}-*pfkB*, pHERD20T-*pfkA*-*pfkB*^{GGM+HRD+DFG} and pHERD20T-*pfkA*^{GGM+HRD+DFG}-*pfkB*^{GGM+HRD+DFG}, mutant *pfkB* and *pfkA* with were amplified using above mutants as templates, and fused with these primers, and *pfkA*-*pfkB*-linker-F/R, then ligated into pHERD20T plasmid.

Primers used for cloning *Shewanella* W3-18-1 KKP_{sw} components and mutants

pHERD20T-2208-F	aggagatatacataccatgTTTCAAAGGCTATTGCAAAAA
pHERD20T-2208-R	acgacggccagtgccaagcttCTACTTGGAATCATCATCTTCAACCT
pHERD20T-2209-F	aggagatatacataccatgATGATTCCAAGTAGATATGAACTCTG
pHERD20T-2209-R	acgacggccagtgccaagcttTCATGAAACCACCTCTGGGTTAG
pHERD20T-2210-F	aggagatatacataccatgATCACATCGAATACTCTGATCG
pHERD20T-2210-R	acgacggccagtgccaagcttTTAATTTAAGATAATGACTGGGTGAGC
pHERD20T-2210 ^{GEA} -F	GCTATGTTGAGGGTTTGA AGCATAGTC ACTATATCCGTTTTGAAT
pHERD20T-2210 ^{GEA} -R	ATTCAAAACGGATATAGTGACTATGCTTCAAACCTCAACATAGC
pHERD20T-2210 ^{HRD} -F	CACACATTCAAGGAGTAATA TTCGAAAAT TTGAAGCCAAGTAATA
pHERD20T-2210 ^{HRD} -R	TATTACTTGGCTTCAAATTTTCGAATATTACTCCTTGAATGTGTG
pHERD20T-2210 ^{DFG} -F	TCGTCGGTAAAAGTTGCAAT TGCATAATT GGTAATCTTTAGCTCT
pHERD20T-2210 ^{DFG} -R	AGAGCTAAAGATTACCAATTATGCAATTGCAACTTTTACCGACGA

Construction of pHERD20T-based constructions for expression of KKP_{sw} components and mutants

pHERD20T-2209 ^{HRD} -F	TACATAAGGCAGGAATTATT TCGAAAAT ATCAAACCAAACAATA
pHERD20T-2209 ^{HRD} -R	TATTGTTTGGTTTGATATTTTCGAAAATAATTCCTGCCTTATGTA
pHERD20T-2209 ^{DFG} -F	CGGAGTTATCAAGGTCTTT AATTATGCA CTTTCGAGAAAACCTTGA
pHERD20T-2209 ^{DFG} -R	TCAAGTTTTCTCGAAAGTGCATAATTAAAGACCTTGATAACTCCG

Primers used for cloning KKP components from other bacteria

pHERD20T- <i>RS13785</i> -F	aggagatatacataccatgCAAGATATATTAACAAAAAGGCTAA
pHERD20T- <i>RS13795</i> -R	acgacggccagtgccaagcttTTATTTACAGCGAATAATTGGAAATGC
pHERD20T- <i>STY4824</i> -F	aggagatatacataccatgTTTACAGAACGACTTGCTCGC
pHERD20T- <i>STY4822</i> -R	acgacggccagtgccaagcttTCAATCTTTCCAAAGAATTACAGGG
pHERD20T- <i>RS23220</i> -F	aggagatatacataccatgAGCCGTGATTCTTACGAAAT
pHERD20T- <i>RS23210</i> -R	acgacggccagtgccaagcttCTACTTAGCCTTGATAACTGGATGAGC
pHERD20T-3792-F	aggagatatacataccatgAGCCGTGATTCTTACGAAAT
pHERD20T-3794-R	acgacggccagtgccaagcttCTACTTAGCCTTGATAACTGGATGAGC
K ^{GFI} KP _{EC039} -F	GATACTAGACTAGCTTAT GACTATGCT CGTATACCAAACCAACCACAAG
K ^{GFI} KP _{EC039} -R	CTTGTGGTTGGTTTGGTATACGAGCATAGTCATAAGCTAGTCTAGTATC

Construction of GFI mutants in PfkA homologues in the synthesized pHERD20T-KKP plasmids with different resources.

Plasmid verification primers

pEX18Ap-F/pEX18Gm-F	AATCTTCTCTCATCCGCCAAAACA
pEX18Ap-R/pEX18Gm-R	CGCCCAATACGCAAACCGCCTCTC
pHDDR20T-F	ATCGCAACTCTCTACTGTTTCT
pHDDR20T-R	TGCAAGGCGATTAAAGTTGGGT

Verification of constructions used for gene knockout and expression

QPCR primers

<i>pfiA</i> -qF	ATGCGGCTGACCTGGATT
<i>pfiA</i> -qR	TGAGCGAACCTCCTGGAAA
<i>pfkA</i> -qF	CGACCTGAAACCAACAAACGT
<i>pfkA</i> -qR	TTAAACCATTTGCTGAAAGGGAA
<i>pfkB</i> -qF	GAAATGAGGTGCGTCGGTAT
<i>pfkB</i> -qR	ACAAGAATATATCTACGGCAATGAC
<i>pfpC</i> -qF	GCCATGACAACTGCTCCACT
<i>pfpC</i> -qR	TTTTCTATGCTCGCTTGCTTT
<i>gyrB</i> -qF	CAAGTACGAAGGCGGTCTGAAG

Quantification of Pf4 and Pf6 phages in effluents and expression levels of KKP_{MP} components in MPAO1 biofilm at different days

<i>gyrB</i> -qR	GCAGAGCAGGTTCTCGTTGAA
<i>16SrRNA</i> -qF	TGGTTCAGCAAGTTGGATGTG
<i>16SrRNA</i> -qR	GTTTGCTCCCCACGCTTTC

References

- 1 Chandler, C. E. *et al.* Genomic and phenotypic diversity among ten laboratory isolates of *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* **201**, e00595-00518 (2019).
- 2 Burkinshaw, B. J. *et al.* A type VI secretion system effector delivery mechanism dependent on PAAR and a chaperone-co-chaperone complex. *Nat. Microbiol.* **3**, 632-640 (2018).
- 3 Li, Y. *et al.* Excisionase in Pf filamentous prophage controls lysis-lysogeny decision-making in *Pseudomonas aeruginosa*. *Mol. Microbiol.* **111**, 495-513 (2019).
- 4 Hoang, T. T., Karkhoff-Schweizer, R. R., Kutchma, A. J. & Schweizer, H. P. A broad-host-range Flp-*FRT* recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. *Gene* **212**, 77-86 (1998).
- 5 Qiu, D. R., Damron, F. H., Mima, T., Schweizer, H. P. & Yu, H. D. P-BAD-based shuttle vectors for functional analysis of toxic and highly regulated genes in *Pseudomonas* and *Burkholderia* spp. and other bacteria. *Appl. Environ. Microbiol.* **74**, 7422-7426 (2008).