

Supplementary Material

Novel immunity proteins associated with colicin M-like bacteriocins exhibit promiscuous protection in *Pseudomonas*

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Plasmid/strain	Purpose of use	Reference					
pET28a	pBR322 origin, His-tag/thrombin/T7 tag : Km ^R	Novagen					
pJB3Tc20	Broad-host-range cloning vector: Ap^{R} , Tc^{R}	(Blatny <i>et al.</i> , 1997)					
pCMPG6248	pET28a with 827-bp PCR-amplified fragment containing <i>pseuM</i> from This study						
r	<i>P. svringae</i> DC3000, cloned in NcoI/XhoI						
pCMPG6250	pET28a with 866-bp PCR-amplified fragment containing <i>pseuM</i> from	This study					
1	<i>P. aeruginosa</i> NCTC10332, cloned in NcoI/XhoI	<i>,</i>					
pCMPG6251	pJB3Tc20 with 445-bp PCR-amplified fragment containing <i>pmiA</i> from	This study					
	<i>P. syringae</i> DC3000, cloned in PstI/XbaI						
pCMPG6252	pJB3Tc20 with 467-bp PCR-amplified fragment containing pmiA from	This study					
-	P. aeruginosa NCTC10332, cloned in Pstl/EcoRI	-					
pCMPG6266	Mutant construct of pCMPG6251; D36A variant of the encoded PmiA	This study					
pCMPG6267	Mutant construct of pCMPG6252; D34A variant of the encoded PmiA	This study					
	ID 2T - 20 - 1/1 504 ha DCD 1'C - 1 for some for the initial states of the	TTL: (
pCMPG6269	pJB31c20 with 504-bp PCR-amplified fragment containing <i>pmiA</i> from	This study					
"CMDC(270	P. fluorescens Q8r1-96, cloned in Pstl/Xbal	This study.					
pCMPG6270	Mutant construct of pCMPG6269; D39A variant of the encoded PmIA	This study					
pCMPG6271	pET28a with 812-bp PCR-amplified fragment containing <i>pseuM</i> from	This study					
petiti Goz/T	<i>P. fluorescens</i> O8r1-96, cloned in NcoI/XhoI	iiio otaay					
pCMPG6272	pJB3Tc20 with 478-bp PCR-amplified fragment containing <i>pmiA</i> from	This study					
penn colle	Pseudomonas sp. 5. cloned in PstI/XbaI	11110 00000					
pCMPG6273	pJB3Tc20 with 465-bp PCR-amplified fragment containing <i>pmiA</i> from	This study					
1	Pseudomonas sp. Pf153, cloned in PstI/XbaI	5					
pCMPG6274	pJB3Tc20 with 459-bp PCR-amplified fragment containing <i>pmiA</i> from	This study					
	P. fluorescens Pf29Arp, cloned in PstI/XbaI						
pCMPG6275	pJB3Tc20 with 497-bp PCR-amplified fragment containing pmiA from	This study					
	P. brassicacearum DF41, cloned in PstI/XbaI						
pCMPG6276	pJB3Tc20 with 466-bp PCR-amplified fragment containing pmiA from	This study					
	Pseudomonas sp. GM21, cloned in PstI/XbaI						
pCMPG6277	pJB3Tc20 with 456-bp PCR-amplified fragment containing pmiA from	This study					
	P. fluorescens AU2390, cloned in PstI/XbaI						
E. coli BL21(DE3)	Expression host	VWR					
E. coli DH5a	Propagation of pET28a- and pJB3Tc20-derived plasmids	Thermo Fischer					
P. aeruginosa	Indicator strain for PaeM (PseuM _{NCTC10332})	J. P. Pirnay					
CPHL12447		DCCL					
P. aeruginosa	Strain encoding PaeM (PseuM _{NCTC10332})	BCCM					
NCIC10332							
P. jiuorescens F113	Indicator strain for PfIM (Pseu $M_{Q8r1-96}$)	(Kedondo-Nieto <i>et al.</i> , 2012)					
D fluomagaana 00-1 06	Stroin anading DfIM (DecuM	2013) (Loper et al. 2012)					
r. juuorescens Qor1-96	Sham encoding Phivi (Pseuvi _{Q8r1-96}) Indicator strain for DavM (DsauM	(Loper <i>et al.</i> , 2012)					
<i>F. syringae</i> DC2000	Stroin encoding DavM (DeavM	$\frac{DUUM}{(Dual1 at al 2002)}$					
1. synnigue DC5000	Suam chooling r Sylvi (r SculviDC3000)	(Duen <i>et ut.</i> , 2003)					

Supplementary Table S2. Primers used in this study.

Number	Sequence ^a (5' to 3')	Purpose of use
PGPRB-8461	GCTCACTCATTAGGCACCC	Sequencing of inserts in pJB3Tc20
PGPRB-9001	TGGCTA <u>CCATGG</u> GTCCTATTGAGCTTCCTCCGACATACAT	Cloning of PseuM _{DC3000} in pET28a (pCMPG6248)
PGPRB-9002	TGGCTA <u>CTCGAG</u> GTTGCCACTAACCGTAACCGG	Cloning of Pseu M_{DC3000} in pET28a (pCMPG6248)
PGPRB-9003	TGGCTA <u>CCATGG</u> GTGCAATGGATCTTGGTACCACAACAAT	Cloning of PseuM _{NCTC10332} in pET28a (pCMPG6250)
PGPRB-9004	TGGCTA <u>CTCGAG</u> ACCAGAAATATTAACAGGGATAGTACCC	Cloning of PseuM _{NCTC10332} in pET28a (pCMPG6250)
PGPRB-9005	TGGCTA <u>CCATGG</u> GTGAATTCGAGCTTCCAGCTACTT	Cloning of Pseu $M_{Q8r1-96}$ in pET28a (pCMPG6271)
PGPRB-9006	TGGCTA <u>CTCGAG</u> TCGGGCGTAGCTAATAGGG	Cloning of Pseu $M_{Q8r1-96}$ in pET28a (pCMPG6271)
PGPRB-9007	TGGCTA <u>CTGCAG</u> CTTTCCATAAGGATTGACAATGGCTGC	Cloning of PmiA _{DC3000} in pJB3Tc20 (pCMPG6251)
PGPRB-9008	TGGCTA <u>TCTAGA</u> AGCGCCTGAAATCAGACGCTA	Cloning of PmiA _{DC3000} in pJB3Tc20 (pCMPG6251)
PGPRB-9009	TGGCTA <u>CTGCAG</u> ACCATAAGAGGATGGTTTCATGAGA	Cloning of PmiA _{NCTC10332} in pJB3Tc20 (pCMPG6252)
PGPRB-9010	TGGCTA <u>GAATTC</u> TTGTATCGCAGCTTTACGTCTACT	Cloning of PmiA _{NCTC10332} in pJB3Tc20 (pCMPG6252)
PGPRB-9011	TGGCTA <u>CTGCAG</u> AGGTTTTTGCAATAATATCGCTAGGAT	Cloning of PmiA _{Q8r1-96} in pJB3Tc20 (pCMPG6269)
PGPRB-9012	TGGCTA <u>TCTAGA</u> CATCATCCTGCGCAGTCATG	Cloning of PmiA _{Q8r1-96} in pJB3Tc20 (pCMPG6269)
PGPRB-9013	CTACAGCCTTCTGCTACCTATGGGCTG	Construction of a D36A mutation in PmiApc3000 (pCMPG6266)
PGPRB-9014	CAGCCCATAGGTAGCAGAAGGCTGTAG	Construction of a D36A mutation in PmiApc3000 (pCMPG6266)
PGPRB-9015	GTCAACCCCGATGCCACCACGGGTGTT	Construction of a D34A mutation in PmiANCTC10222 (pCMPG6267)
PGPRB-9016	AACACCCGTGGTGGCATCGGGGTTGAC	Construction of a D34A mutation in PmiANCTC10332 (pCMPG6267)
PGPRB-9017	AACCCAAGTGCTACATCTGGC	Construction of a D39A mutation in PmiA _{O8r1.96} (pCMPG6270)
PGPRB-9018	GCCAGATGTAGCACTTGGGTT	Construction of a D39A mutation in PmiA _{O8r1-96} (pCMPG6270)
PGPRB-10029 PGPRB-10030	TGGCAGCAGCCAACTCAGCTT	Sequencing of inserts in pET28a Sequencing of inserts in pET28a
PGPRB-10318	TGGCTA <u>CTGCAG</u> CGATAGGCATAGAGAAGACATGAA	Cloning of PmiA ₅ in pJB3Tc20 (pCMPG6272)
PGPRB-10319	TGGCTA <u>TCTAGA</u> GCGGATGGATTGGTTGATT	Cloning of PmiA ₅ in pJB3Tc20 (pCMPG6272)
PGPRB-10320	TGGCTA <u>CTGCAG</u> TTTCTCTAGGTGAACAGCAATGGTAG	Cloning of PmiA _{Pf153} in pJB3Tc20 (pCMPG6273)
PGPRB-10321	TGGCTA <u>TCTAGA</u> CCGCTCGTCCTGCAAAAGGC	Cloning of PmiA _{Pf153} in pJB3Tc20 (pCMPG6273)
PGPRB-10322	TGGCTA <u>CTGCAG</u> TTTTTCAAGGTAACCCACTATGATT	Cloning of PmiA _{Pf29Arp} in pJB3Tc20 (pCMPG6274)
PGPRB-10323	TGGCTA <u>TCTAGA</u> TGCCCTGGGAAGGACAAG	Cloning of PmiA _{Pf29Arp} in pJB3Tc20 (pCMPG6274)
PGPRB-10324	TGGCTA <u>CTGCAG</u> TTTCTCTAGGTAAGTAGCCATGG	Cloning of PmiA _{DF41} in pJB3Tc20 (pCMPG6275)

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PGPRB-10325	TGGCTA <u>TCTAGA</u> AAGAGTAATCTTCACCGCGCA	Cloning of PmiA _{DF41} in pJB3Tc20
		(pCMPG6275)
PGPRB-10326	TGGCTA <u>CTGCAG</u> ATAACAGGAGCAACACTCCATG	Cloning of PmiA _{GM21} in pJB3Tc20
		(pCMPG6276)
PGPRB-10327	TGGCTA <u>TCTAGA</u> GCCTAAAGATGTTCAAGGGAGT	Cloning of PmiAGM21 in pJB3Tc20
		(pCMPG6276)
PGPRB-10328	TGGCTA <u>CTGCAG</u> AAGAGCGTGGAATATTTAAATGAATCT	Cloning of PmiA _{AU2390} in pJB3Tc20
		(pCMPG6277)
PGPRB-10329	TGGCTA <u>TCTAGA</u> AAACTCATGATCGAGCGTACT	Cloning of PmiA _{AU2390} in pJB3Tc20
		(pCMPG6277)

^a Restriction sites incorporated in the primers are underlined: GAATTC, EcoRI; CCATGG, NcoI; CTGCAG, PstI; TCTAGA, XbaI;CTCGAG, XhoI.

Figure S1. (A) Multiple sequence alignment of ColM domains derived from unique PseuM proteins from *Pseudomonas* spp. Abbreviations for species names are as in Figure 1. Other abbreviations: Pced, *Pseudomonas cedrina*; Pcor ory, *Pseudomonas coronafaciens* pv. *oryzae*; Pden, *Pseudomonas denitrificans*; Pkor, *Pseudomonas koreensis*; Ppro, *Pseudomonas protegens*; Psyr ace-cori-mors-syrthe, *Pseudomonas syringae aceris-coriandricola-morsprunorum-syringae-theae*; Pthi, *Pseudomonas thivervalensis*; Pver, *Pseudomonas veronii*. Grey shading reflects the degree of conservation. (B) Unrooted maximum likelihood phylogenetic tree of ColM domains of pseudomonads (black) from panel (A) and previously characterized ColM domain-carrying bacteriocins from other β - and γ proteobacteria: colicin M (ColM, red) from *E. coli*, pectocin M1 and M2 from *Pectobacterium* spp. (PecM1/PecM2, pink), burkhocin M1 and M2 from *Burkholderia* spp. (BurM1/BurM2, grey). *Pseudomonas* abbreviations as in (A). The clade of *Pseudomonas* ColM domains from bacteriocins linked with a *pmiA*-type immunity gene and *pmiB*-type immunity gene are in a red arc and a blue ellipse, respectively. Other abbreviations: Bamb, *Burkholderia ambifaria*; Ecoli, *Escherichia coli*; Pcar, *Pectobacterium carotovorum*. The scale represents 0.6 substitutions per site. Bootstrap values (percentages of 1000 replicates) are not shown for visibility.



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	1 10		20	30	40	50	60	70	80	90	100	110	120	130		140	150	160	168
Pout ABAC63	FYEPPLMELAGT	YMMER	RTRYVNIC	SIGRAMVPSD	FKE-MMD	A -ATKG PGT	YAT - DOFE YN	REMIN-LYN	AGLICERINSG	NMET	INTGSDESWO	DEYYTLKPD	ANDADHSIN	TYMORKIND	TREME	D-RFGYNN	VERSOR	BFSGRK	,
Pced BS2981	FYEPPLMELAST	GAWAYE	DORNVHIA	SINCLARD	FOR-TAK	-NDPNNLGT	FEIN-EPFSYN	DKAPIDVPA	AGMINGRAS	NAKET	IVIOSD ET YG	NESYSLARD	MDADKSM-	TWAODALI	LKGIGE	E-SFGHND	NILEEOA	DYSGTR	
Psp Os17	FYEDPLMEMONI	GWIFG	TRNVRIE	SINDOMVATD	FKE-WN	INBAKGAGA	YRIK-EPFS YN	DKAPIDLPA	AGMICRES	EMET	LELGPN OF YS	NGVYRLNPD	LYDAGSEN-	TPAODGLIT	LRGIGE	E-TFGHTDMB	NIVEEKT	FSGGR	
Ppro K94.41	FYCDPLMEMONI	GWIFG	GVTRNVR	SINCOMVATO	FK - IVN	INPDKGAGA	YRIK-EPFSYN	DKAPIDLPA	AGMIGRISG	EMEGI	LELRPN EF YS	NGVYRLNPD	LYDAGSEN-	TPAODGLER	ELRR	E-TFGHTD B	NEVEEKT	BF SG GR	
Pcor ory ICMP9088	FYEPPMMEVVII	GWIFG	GODRNVHIG	SINTIMUATO	FT - TG	I NNAANGAGT	YQLQSAPFSYN	NKAPLDLPA	AGMIGR VS G	NEGV	INISADEGYS	SSYTLNSD	LYNADMSN-	SWLODSLIN	TISSIGI	D-AFGHTDMK	NFLDSQS	FTGNR	
Psyr the ICMP 3923 (1)	FYGIPMMPIVAI	GWIFC	DDRNVHIG	SIGUTMVATD	FT - TD:	INNAVNGAGT	YQLQSAPFSYN	NRAPLDLPA	AGMIGRVSG	NETEV	ITITAD GYS	SESYTLIPD	LYNAD SN-	AWWORSDIN	FLSSIGI	D-AFGHTDMK	NFLDSQS	FTGNR	
Psyr mors M302280 (1)	FYGIPMMEIVAI	GWIFG	GDRNVHIG	SIGLIMVATD.	FT - TD	INNAVNGAGT	YQLQSAPFSYN	NRAPLDLPA	AGMIGRVSG	IN III CV	TITADEGYS	SSYTLIPD	LYNADMSN-	SWWQESHIT	FIGSIGI	D-AFGHTDHK	NFLDSQS	FTGNR	
Psyr USA007	FYSSPVMPIANI	AWEFG	GEDRNVNIG	SINCOMVLSD	FKP-TTD	VINPATVSGC	YAID-APFS YN	SKAPINLPV	AGVEGRUNG	NMBGT	IVINSDNTIS	TGHYMLNKD	TYDADESN-	SYWQDALIN	FLRGEGI	D-TFGRTD T	NUISTQP	VFF G RK	
Psyr ace M302273	FYCSPIMPIANV	AWUFG	GADRNMNIG	SINDOMVLSD.	FKP-IID	V NNPATAPGV	YPID-APFS YN	SKAPINLPV	AGV EG R VS G	SN IIG CH	IVINADNTYT	TGQYVLNKD	VYDADASN-	SYWQETHE	FIHGIGE	E-VFGHVD T	NUIGAQP	VFSGRK	
Psp 5	LSGDIFTPLKAF	AFFIYG	GATATVPLE	KTR U SPSP-Q	KIPELQKI	MOSA-AICKT	ED-DIRVI	TTFQDS FNT	GAYINGNUM	RUIGT	IE INKE KVT	S GQA RAWND	IYDGNASTH	QIWADTEL	ALRLMGE	E-KNKATHME	EMLESIP	FYSN	
Psp 2(2015)	FSCGIFTATKAI	ANAMYC	GAKATVSUK	DIGUS PSV-E	KUREUKSI	LECAN-EIGST	QU-DINVI	YTGQDSFAAD	RTYIKCNIN	RHIGT	IT DSE OVVS	SGQARAWND	LYDAEASDH	IPLDDSAUM	ALRS MET	K-VTNAVS ME	EMPESLP	NYSTTP	
Psp p21	F. SICGWE THE RELL	AWULS	GOOAK WN UN	NIG APEL-H		PARA-SAGIS	RU-DVTE	WN TIGM DS NIS	RIYIGSINN	RMIGD			KYDAWASSHI	GGE DE KALIN	LIREMAN	R-VANAQDE	L HEBDLP	NIAN	
Pflu Q8r1-96	P SIGGTP THE NAL		STAS VUIS	KIGINPTP-E	KING DIMA	I HNTA-RISET	I.M.M. TRUE	THEQUENTPI TOTOLOGY THE	KT I III FI IIIII					IGF DDAAD	LIKE Mot	K-VANAKDNA	VENELLP	DIAK	
Pbre BS2//1	F SEVVETER AND		Z DUCUDIO	DTGERPTP-D	ALLEDINA.		D M M D N				VIVATIS SAVI			AQEDUGAUS	TRONGE		NET SALP	NEOR	
Pilu Pi29Arp	L SIGGTL THE LAT			PICE PAP-A	KINDIMA.						VI IVIS DILKI	A TTURNED	DYDAMA SSLI	ALF DE GAL BE	TIPENCE		DETERTO	NESP	
Pep Df153	FSHATEPHEKNE		REVENDER	PHETT PAP-N	KINDIMA	TRITALCVEVE	TR-NT.KVI	STAO BENUZ	PTVICATION		WTRVTS PT.K	A CLUERADS	DVD AMA SISILI	AAFDOHADS	TIPE	-VANARDER	DEPERT. D	NFCR	
Pbra DE41	FSECTETEEKAF	SMAL	CONTRACTOR OF	RIG SPTP-S	KIPDIMA	TINTALGVEVT	SM-MT.KM	NS TAO DENVAL	RTYIKCNUM		VT BYAS BPLK	SEKTIMAN	RYDAMASSHI	AAFDOALS	TREMOT	R-VANARD E	RETERLP	NFSR	
Psp NFACC04-2	FSEGIFTEFKEF	SWALE	REVEVER	RIGISPTP-SI	KIPDIMA	INTA-GVEVT	SM-NLKM	STAO DENVAL	RIYICONT		VT NYAS BELK	SCHIMANSD	RYDAMASSH	AAFDOOADS	LRENGE	R-VANARD E	RATELP	NFSR	
Psp NFACC14	FSEGIFTPFKAF	SIMILE	REVISVRID	RIGISPTP-S	KIRDIMA	INTA-GVEVT	SM-NLEMI	TAO DENVAL	RIYICANI	Dil Co	VT TYAS BLLK	SEKIKAWSD	RADAMASSH	AAFDDOADS	LRENGE	R-VANARD E	RITELP	NFSR	
Psyr GR12-2	ISCGILSEMANE	GIFFNE	CAOVITNIN	NES NESO-SI	TRAILEA	AFTAA-IIESS	PISISKAL	MUTSDSFWIT	GAWINGNI	KIESV	LD FHD RLS	ASARAMND	TEDENASMH	SGFACELIR	GRL	E-SMS SOPME	ADDELP	FSTHR	
Psp BRG-100	ISASELGEVSEL	GIENNE	GATVRMDUT	KIGLNPSV-T	KIPMLEQ	QFSAL-SIGIT	RUVLDKVI	YNTEDDSENT	GSVICONTIN	KIEGN	ATNYEGAVV	NGEA RAYND	KYDANQGEF	NALPDAAR	LRADON	N-GTGAQE	ERSLP	DIHR	
Pver R4	ISATELGEVSAL	GIERNE	GATVRMDUT	KIGLNPSV-T	KIPMEEQ	2FSAL-TVETT	RVLDKVI	YNTEDDSENT	GSVIGNITI	KIECN	ATAYENSVV	NGEARAYND	KYDANQGEF	NALPEAAL	/LRA DI	N-GTGAKE EE	ERRSLP	DIQR	
Ptol 6264	ISATELGEVSAL	GIFING	GATVRMD	K GLNPSV-T	KIPMLEQ	QFSAL-TVEIS	RUVLDKVI	YNTEDDSENT	GSVIGNIAL	KIECN	ATAYENSVV	NGEARAYND	KYDANQGEF	NALPEAAL	/LRAIDN	N-GTGAKE E	ERRSLP	DIQR	
Psp GM21	LSCGLLTVFATF	GFFFG	GATAETNIN	SIGEKESS-T	FIPMLES/	A INA-PT CT	AUVLDKVI	YNTGS	EMWINGNI	KIECT	IM HES KLS	NGGARSYND	IYDANPEDF	SAIGDSAIR	/LAAMEP	K-YLNAQP 👥	AKETNV	IFTER	
Pput RW10S2	FSGGVLTPVAAL	GIFIYG	GOQASTSIN	SIGLNINA-T	PIPALDQ	ALISS-SVESS	RIVLDKVI	YNTANDSWLT (GLWICHII	KIES	VINADERIS	DGEARAYND	IYDANPGEW	SAFGESALQ	/LSE DF	R-HLEAENMA	EKRAYP	BLQK	
Psp NFIX28	LSCGLFSEVKEM	ENY	GERMINVIDUN	SUDUN V KS-N	SUPLUTQ	HOFSNTPETY	HUSDPKV(MATED DSLAT	GSLINGRIM	NMEGD	FT HEN GIWS	T DA RAWHD	VEDENASN	GVVADIALI	LGAAFNO	GTPRE	DUYUTHQ	NLVGAGNAP	LD
Pflu HK44	LSCGTETEVKEE	THYPWG	GEKLESVNUN	NUGUN IRA-H	CHELMAN'	THASTREASSY	RESEPKVA	DTRH DSAITA	AAYIKGRUMI	KAEGD	FTHHTNESWIT		TADEMESIM	STLLETEES	AGEVIBO		REFERIN	HLSGIGEOP	N
PSp GM55	L SEGTE SEVINEE	AHYHWE		NEDEN ERA-SI	CIEL AN.	THASTKES SKI	Y DE DET	DIRI DERIT			FT MEEN SSWI			STLLET	AGEVISO		DELERK	RENGICEVE	DLQ
PIIU AUTITI4	T STATE OF T L STATE	A STATE OF		NICONMOR-N	REAL PAIN.		V S P PKTI							STALLIN	COVESC		DEFENCY	DINGAGINE	13 E
	LSCHETCSELLE			NUCINIKA -N	REPT DAS		V S P PKTI		CA VICOTAT				TYDE DA SML	STADEVELT	COVESC		DEFERUX	PISCACEUP	T D
Pen H1h	LSCOTFSELKER	ATYNG	CERMEVNIN	NICONVSA-N	FIPLIAS	A STREA STV	VISIPKTI	A TIN DSNUT	SAYINGRUM		FTIDKTESWVI	DEVIRAMED	TYDF DA SM-	STALELUC	GRVFSC		DEFEDHK	RISCICENE	AP.
Psp CRS05-R5	LSCGTFSEIKAF	AWW	GRIRVNIN	NIGINVSA-N	FIPLIAS	ASTREACTY	YISDPKTI	ATTNDSNVT	GAYIGRITH	KARE	IFT DNA SWV	DEVIKAWITD	TYDEDASN	STALPLUT	AGRVFSC	THME	DIFERHK	RLSGTGEHP	TP
Pkor BS3658	LSCGTESPIKAE	ANYWE	ERLEVIN	NIGINVSA-N	ELPLIAS	ASTREACTY	YNSDPKII	ATINDSNUT	GAYICRIM	KTERN	FT DNA BWV	DEVIKAMTO	TYDEDASN-	STALELLE	AGRVFSC	GTHME	DIFERHK	RLSGTGFHP	TP
Psp NFACC51	LSCGTFSEVKAL	ATYWE	GEKLEVDIN	TIGEOIRE-H	KESLEKO	TESTKSPETY	HOVDPKVA	DTGRDSLIS	GAYICRIM	HMECO	FTHODTSWO	LGAVKAVHD	FYDFDKSD-	PLALOOLIN	AGRADO	GTGME	NESCHK	HLIGKGEOT	PNE
Pthi PITR2	L SCGTF SEVKAL	AYWG	GEKLEVDIN	TIGEQIRE-H	KESLEKQ	TESTKSPETY	HEVDPKV7	DTGKOSLIS	GAYICRIM	RMEGD	FTENODTSWO	LGTVKAYHD	FYDFDKSD-	PLALEOLO	AGRADPO	GTGME	NESCEHK	HUIGKGEKT	PNE
Psp GM33	LSGGTFSPVKAL	GIYIWG	GOKLEVDIN	TIGLNILE-H	KEPLERQ	ALEHTKLPEVY	NOVDPRVO	YDTYS DNLAT	WAYIGRIM	S Mr CD	FISNDDSWR	IGVIKAYHD	RYDFNESD-	PPALCHEIT	AGRADPO	GTGEE	DESEEHK	HIVGKGHQV	OPE
Psp GM49	LSCGTFSEVKAL	GIYIWG	GOKLEVDIN	TIGENTLE-H	KLPLLRQ	ALEHTKLPDVY	NEFDPRVC	COTFN DNLAT	WAYIGRIAN	5 Mr GD	FISNDDSWR	IGVIKAYHD	KYDFNESD-	RPPALCHICT	AGRADPO	3 T G B E	DISEEYK	HIVGRGHQV	QPE
Paer AZPAE14373	FNGGVYTPAAAF	AYTG	GKKMNFPIE	RENEKPNV-K	AMPOFIG	VETSS-PMCQT	TZ-DFNVI	YATAKDSWVA	GNTWGELTL	RIVEI	LV STS COWN	RGEIRAYDD	LYDFNPSNH	RIETARGMERI	LGRENGS	ZKFKDT T P M P	GUPBAIP	VNISG	
Paer JJ692	FNEGVYTRAANE	AYTIC	GKKMNFPUE	RUNKPNV-K	AMPOFIC	VET SS- PMCQT	TM-DFNVI	ATAKDSWVA	GNTMCELLIN	RUVEI	LV MSTS EDWS	REEIRANDD	LYDFNPSNH	TETADGMURI	LGREMG	QKF KD T T P M P	GUPBAIP	NISG	
Psp HMSC067G02	FNCGVYTEAATE	ANY	GKKMNFPUE	RUNEKPNV-K	AMPOFIC	VETSS-PMC/T	TM-DFNVI	YATAKDSWVA	GNTWCELIN	RIVGI	LV MSTS COMS	RGEIRANDD	LYDEMPSINH	TETADGMIRI	LGREMG	2KFKDTTPPP	GUPBAIP	NISG	
Pden 481_PDEN	F MCGVYTEAAME		GREMINEPUE	RENEKPNV-K	AMEOFIC'	THE SSE PMSON	T.M. M. ENVI	A TAK DEWVAR	GNTWGE LINE			RELINAND	LYDENESNES	TETADGMIKI	COREMES	2KFKDTTPPP	GEPEALP	DISG	
Paer AZPAE 14352	F NGGVIIIIAAAE	ハ朝鮮朝日に日か	CANDENE PILE	RUN K DNU-K	AME OF TC.	THE COL DM SOF			CNTWEETHE		TEV MOTO SQUAR			TETALGMENT	CDEME	2NE ND THE HE	CHERNALP	TSG	
Paer NCTC10332	FMACUVTEDANE		KKMNED	DUNKDWA-K	AMPOFIC:	THESS PARA			CNTWEET IN			REFIERMEN		TETADOMEN	CREME	OKEKDT DE DE	CHONTTO	TSG	
Pflu AI 12390	FSCOPLEALHAL	ONAND	GUTVSFPIN	NICKVNL-S	SDAMN	AHSTNPVEKS	T - VKFI	NVTKISVSS	SLVICONTIN	RAZ	VD WYAS BEWS	NATIBANN	RYDAMPSMH	NWLGETTERS	PIGKIMMO	DYEMP	REPERTS	MIMVGO	
Psp AI 19518	FSCOPLEALHAL		GRTVSFPIN	NIGIKVNL-SI	BDAMN	HSANPVEKS	T - VKFI	NVTKDSVSS	GI. VICONT MI	RMZ	VD WAS BOWS	ATRADO	RYDAMPSMH	NWLGDTUIS	TIGKAM	DYEMP	EMPERTS	MIMVGO	
Psyr CC1417	FEGMMTREHAL	ANGMER	CAPVSFPIO	NMGINVDI-R	STROWMNS	DVARPVERS	ST-DVSF7	DVGKOTTSS	TTICNEN	RINCE	ID SSS BAWT	NEETRAMND	VYDANPENH	GWLGDNLIS	INSEFPE	TSMD	EMPESLP	TIGGN	
Psyr cory ICMP 17001	LSGGLMTPFHAL	ARGIER	GAPVMEP	NMGINVDI-R	GIRDMAN	ADSVRPVETS	S -DVNFA	DVGKDSNAS	WLTICHIM	RIVET	TD SSS AWT	SEIRAEND	VYDANPSINH	GWLGENLIS	/ISA PE	FTSMS	EPPSLP	TVSGN	
Psyr syr CRAFRU11	LSGGLMTPFHAL	ANGMER	GAPVMEP	NMGENVDI-R	GIRDAMN	ADSARPVETS	SI-DVNF7	DVGKDSNAS	WLTIKGNIM	RIVET	ID SSS GWT	SEIRAFND	VYDANPSNH	GWLGENIES	/LSAMPE	T S B S	EPESLP	TVSGN	
Psyr syr ICMP 3023	LSGGLMTPFHAL	AGGEG	GAPVMEP	NMGENVDI-R	GIPDVMN	ADSARPVETS	S -DVNF7	YDVGKDSNAS	WLTIGNIAN	RIVET	ID SSS AWT	SCEIRAFND	VYDANPSNH	GWLGDNLIGS	/ISAMPE	T P M 6	EPESLP	VIVSGN	
Psyr syr 642	LSGGLMTPFHAL	AGGIEG	CAPVMEPIQ	NMGLNVDI-R	GIPDVMN	ADSARPVETS	S - DVNF7	YDVGKDSNAS	WLTICN	RIVET	ID SSS AWT	SGEIRAFND	VYDANPSNH	GWLGENLES	/LSAMPI	FTS 26	EPPSLP	VIVSGN	
Psyr cori ICMP 12471	LSGGLMTPFHAL	AGGEG	GAPLTFP	NMGLNVDI-R	DIPDVMN	IDSARPVEIS	S - D VNF7	DVGKDSNAS	WLTICON	RUVET	ID INSS AWT	SGKIRAFND	VYDANPSNH	GWLGENLES	LISAMPI	FTS B 6	EPPSLP	VIVSGN	
Psyr mor M302280 (2)	LSGGLMTPFHAL	AGMEG	GAPLITEPUQ	NMGLNVDI-R	GIRDMMN:	ILDSARPVEIG	S D-DVNF2	YDVGK DSNAS	WLTEGNER	RUVET	ID NAS CAWT	SGEIRAFND	VYDANPSNH	GWLGENLES	LISAAPI	T S M 6	EMPSSLP	M VSGN	
Psyr the NCPPB 2598	LSGGLMTEFHAL	AGMEG	SAPLTFP 10	NMGUNVDI-R	GLEDWMN.	IDSARPVEIS	S VNF7	DVGKDSNAS	WLTIKGNUM	RUVSI	ID MAS AWT	SELIRARNO	VYDAMPSMH	GWLGDNLIS	LISAMPI	TSMS	EUPSLT	M VSGN	
PEVE fom DC3000	LANDER MILLING HAR	A RE - DIR	A PRICEPTO	NEW WINDI - R	- HERE AND AND A	VEDSAREVETS	S R - W VNF7	A DELTAY LAND A STATEMENT A ST	ALC: UNKNOWN	Rec 1997 / 101	THE PROPERTY AND AND A	S REPORTED AND A DESCRIPTION OF THE REPORT O	V CONTRACTOR PORT	A AND CHIMNESS	LINSA IN PT	the second se	■N HEP BIS L P	THE ACTION OF	





Figure S2. Multiple sequence alignment of BmiB (*Burkholderia* spp.) and unique PmiB (*Pseudomonas* spp.) proteins. Grey shading indicates the degree of conservation. Abbreviations for *Burkholderia* species names: Bubo, *Burkholderia ubonensis*; Bcon, *Burkholderia contaminans*; Bamb, *Burkholderia ambifaria*; Bokl, *Burkholderia oklahomensis*; Bcep, *Burkholderia cepacia*; Bcen, *Burkholderia cepacia*; Bcen, *Burkholderia pyrrocinia*; Burk, *Burkholderia* sp. *Pseudomonas* abbreviations are as in Figure 1 and Figure S1. Predicted transmembrane helices are boxed in blue, Sec- or Tat-dependent signal sequences in red and lipoprotein signal sequences in yellow. Two conserved cysteine residues are highlighted in green.

Bubo Bu	MFSTVLCFMMRWILWITAAAIGAFVLLFVWAISRYDRTEG	S DEDAPIEVFRGKILANIED-OGVOSNRIO EDGAPRYHAWKLGVNEFELLVDGT KMLOWID SNEONASY-WRVKESPHAPPS EGN
Bcon LMG 23361	MISKVVRG/LKL/LGLVVAVICAYTLLFGI/MATSRYERTEGS	S 🖉 DMSLDKLREK 🛛 LLRLGE – HGVP – – PGS IR FDGAP 🛛 HAWKLGV 🕮 F 🗳 LR 🗖 AET RMI MMU DONEO MASY – GOT – – – VA PAGO ARKP 🖬 LIOK
Bamb MC40-6	MISRVVRGMLKLMLWLAIATIGAYTLLFILVATSRYERTEGS	S DEDMAIDTLRNK OLLRLKE – HGIP – – PESIR FDGAP ON HAWKLGV DEFULR VEGIRWIZVID ON EQUISSY – DE VROQ PVNQTGQARDR 2010K
Bokl C6786	MMLNRSWKVKV ISVVTLVLVIVGLVIA MYVRVYDGGDG	I 🗃 DMTRQEIKSN 🖬 VK 🗃 A YR - MNKN PK 📈 DEDEDFL 🔂 LNDVA - Q 🗃 K 🕼 🛛 YK 🕼 K 🕅 🖉 K 🕼 🖉 VDN V 🔂 PYD
Bcep DWS 37UF10B-2	MIAEGVLTKKI KYTATASGALLVCASVLFAILVYVRNWDNGDG	P∰EQMSRLEITGFÆEKÆANØ─NGLSKIVEDENFEÆVTDLH-QÆKVEVRSDCHRMIEKÆVELDNVEPYN
Bcen CEIB S5-1	MIABRILIKKI KYTIAAGGALLACALVIFAILA YVRNWDGGDG	P∰RKMTRHEITEFÆRKÆAKØ─MGLSQVIEDENFEEVTDLH-QÆKVEVRSDCHRMIEKKÆRELGIMULDNVEPYN
Bcep DWS 16B-4	MTKAI KYTAAASGALLATALVLFA I SYVRNWDGGDG	P∰RKMSRREITGFNEKNAAKØ−NGSP−−−−QIVEDENFENIPDLH−QNKVNEYRSDCHRMINKNISCINDVEPYN
Bcep ATCC 25416	MISRTMRGMLRLMLWLAGAVICAYTELFIVMAASRYERTEG	S 📴 DMPL DALRKK 🗰 QARLLEAHGIP – PDS IR EDGAP QHAWKLGV 🕮 IELR 🗰 ETRMI IMID 🧾 EQUASY – 🗒 RTIDPLGA BIG QARDRE QH 🕅 K
Bamb AMMD	MTSTAAROMVRIMLASVAVAAATLIAFLVIVGIARYEKDDG	Y DEGASVAELEAKILA BAKE-QHVYPDDAE EVGKPRHADTYGWWGFDLRAPDGNWYDTID DWGRWGF-GKIQKLPLVPATPTQ
Bpyr CH-67	MISTVTRY VRLMLILVATA IALVLEFLIIVGIAR YERDEG	S 😹 DA PARELEAK 🖬 LA 🖬 AKE – QGVQ – – LNE 🕨 E FIGKP R 🗹 Y POKLGW 🖉 AFOLKSREGN 🖉 G 🖬 T 🖬 D 🍺 D R 🖬 T G F – 🎯 K IQ KL PILE PITK PIQ
Bcep GG4	MISTVTRY WRLMLILVATA IALVSLFLVIVGIAR YERDEG	S 💆 🖬 DA PAGE LEAK 🖬 LA 🖬 AKE – QG T Q – – LN D 🛛 E FI G KP RY YADK LG W 🖉 AFDLK SREGN 🖉 G 🛛 T 🖬 D 💆 DL R 🖬 T G F – 🦉 K VQ KL PLE SAK PAP
Burk A9	MISTVTRY WRLWLILVATAIALVSLFLVIVGIAR YERDEG	S 📴 DAPAGE LEAK 🖬 LA 🖬 AKE – QGTQ – – LND 🛛 E FIGKP RY YADKLGW 🖉 AF DLK SREGN 🖉 GTI 🖬 D 🥶 DLR 🖩 IGF – 🥵 KLQ KL PLE SAK PAP
Psp Os17	MERPIAVL ISAAALMVS HGC-DNKKGVG	/ DEAAEQSFFSASDKQHYLD-QNL PVAAESFRLVGTATNDDHNN-WD/VEFDIDGQKEQALLSSDGRUEIT-GR
Pcor ory ICMP 9088	MLKKAVAALMVSSTLA MGCGDQDKVVA-	- DEFINGEAFFKSSIDGYAK-HDKAK-IGNYKIGPGERYDAKNN-WW/VEYSIDGTSYLSTOGTSYCSTOP
Psyr mor M302280	MLKKAVAVLIVSSTLA MGCGDQDKVVA-	- DE TNGE AFFKSSIDG YAK-HDKAK-IGNYKLE PGE RYDAKNN-WYD/VEYSIDGTSYL SWLENGERTELSVEKP
Psyr the NCPPB 2598	MLKKAVAALIVSSTLADVGCGDQDKVVA-	- 📴 RINGEAFFKS S 🖬 DG 🛯 YAK – HD KAK – IGN YKLE PGE R 🖸 DAKNN – W 🕅 / V 🖻 Y S 🖬 D GT S 🖓 L MM S 🖶 DGRT EL S V GK P
Pput ABAC63	MRQGIFIGAALLAA AGC-D-DSGDM	F DE DKGQDFFDQS U KA U FTK—HPPVEGIENVKVLSGATUDTATN—WUVVVEVDVGKDKUNQLUSS EDG HDELS—GR
Pced BS2981	MKKVFGILALFFACVT BACGN-EKQVEN	N <mark>e</mark> sgkgeafftes i na m fsk-hprtgddqsytlqpga rm detnd-w m iv e fdygde r aq allsodg hileis-s r
Psyr ace M302273	MFMLGSKLSPRCIFFIFGLLMGCSEPDLES	- <mark>B</mark> ADRGEGFFTKSINLILLSA-HERSEDLGRFTINGVARIDDRTN-WINLVEFDINGVREVALLSS -DGKI LLS- G RP
Psyr USA007	MLASGGRL IPMCAFFIFGLLGGCGESDLEP	- <mark>B</mark> ADRGE EFFTQS INQMLSA−HDRSADVNRFAMAGTPRMDEHTN-WMMVDEFDIRGQRMLTM LS⊝DGKI LELS-GRP

Figure S3. Multiple sequence alignment of unique PmiA proteins. Predicted transmembrane helices are boxed in blue, Sec- or Tatdependent signal sequences in red. Grey shading indicates the degree of conservation. *Pseudomonas* species abbreviations are as in Figure S1. This multiple sequence alignment was used for construction of phylogenetic tree in Figure 1B.



References

Blatny, J. M., Brautaset, T., Winther-Larsen, H. C., Haugan, K. and Valla, S. (1997). Construction and use of a versatile set of broad-host-range cloning and expression vectors based on the RK2 replicon. *Appl. Environ. Microbiol.* 63, 370-379.

Buell, C. R., Joardar, V., Lindeberg, M., Selengut, J., Paulsen, I. T., Gwinn, M. L., et al. (2003). The complete genome sequence of the *Arabidopsis* and tomato pathogen *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl. Acad. Sci. U. S. A.* 100, 10181-10186.

Loper, J. E., Hassan, K. A., Mavrodi, D. V., Davis, E. W., 2nd, Lim, C. K., Shaffer, B. T., et al. (2012). Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genet.* 8, e1002784. doi: 10.1371/journal.pgen.1002784

Redondo-Nieto, M., Barret, M., Morrissey, J., Germaine, K., Martinez-Granero, F., Barahona, E., et al. (2013). Genome sequence reveals that *Pseudomonas fluorescens* F113 possesses a large and diverse array of systems for rhizosphere function and host interaction. *BMC Genomics* 14, 54. doi: 10.1186/1471-2164-14-54