

Supplementary Material

Exogenous and endogenous phosphoethanolamine transferases differently affect colistin resistance and fitness in *Pseudomonas aeruginosa*

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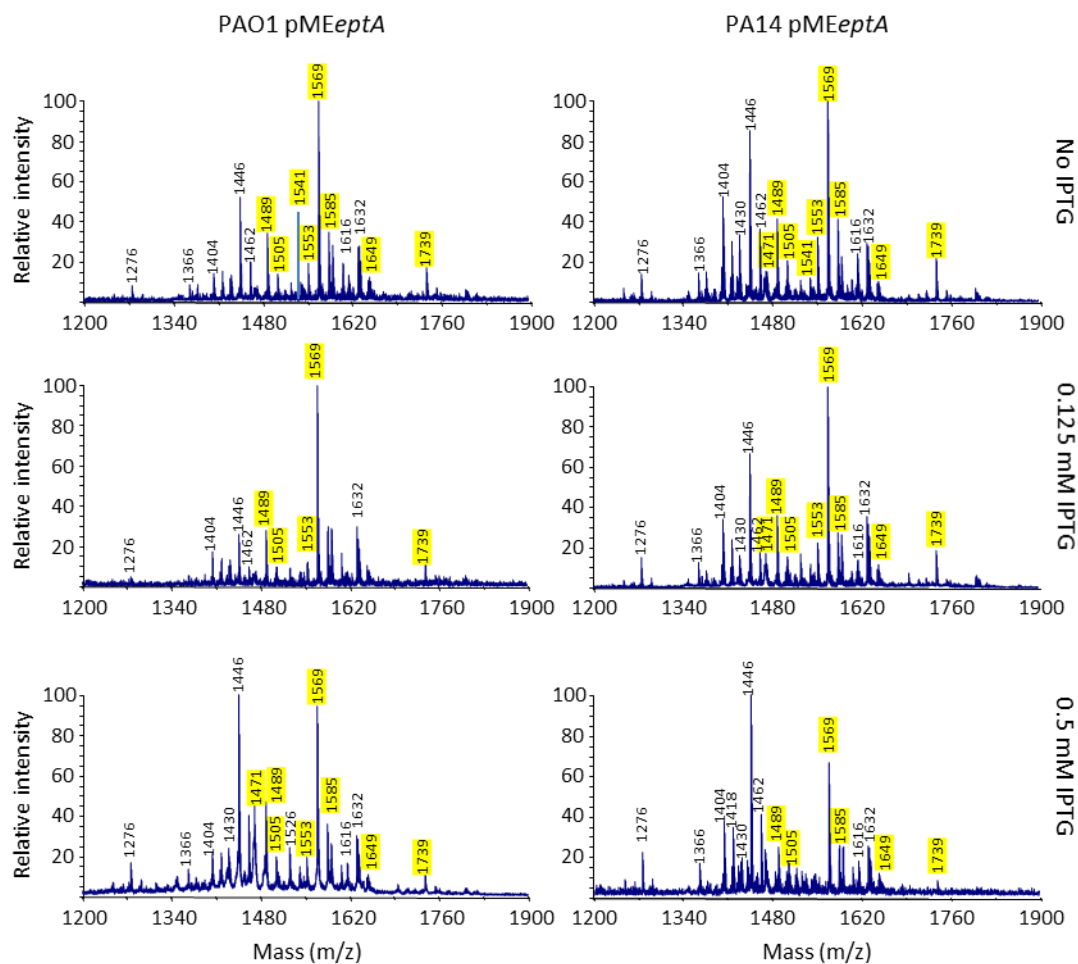
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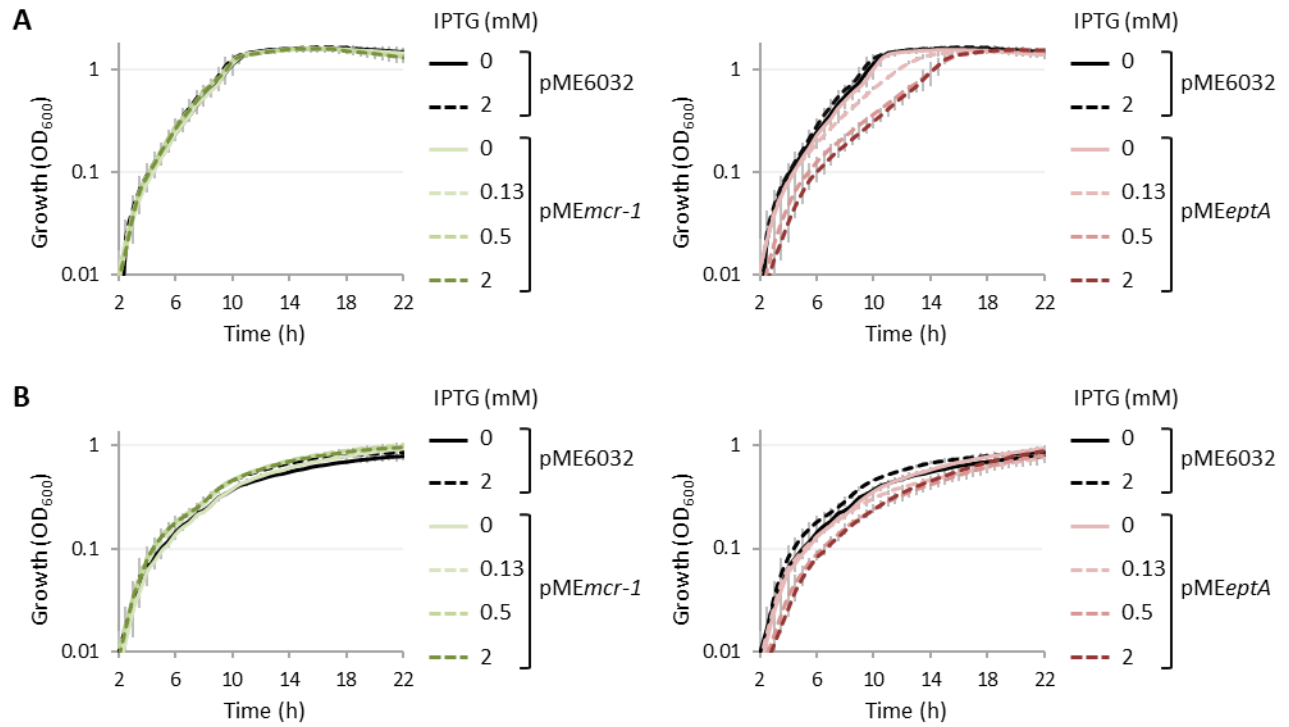
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1. Supplementary Figures and Tables

1.1. Supplementary Figures



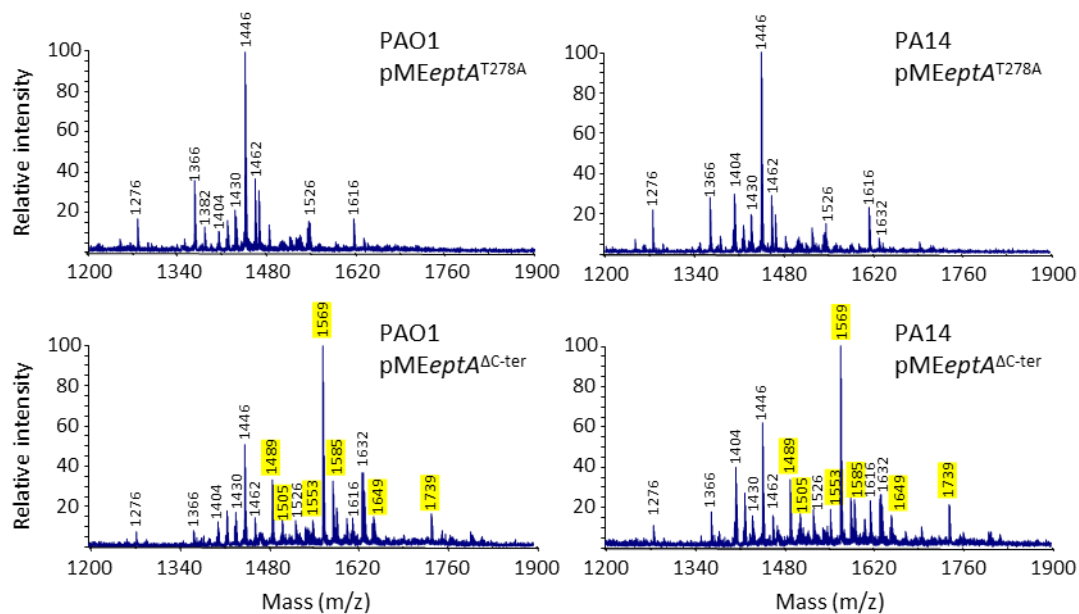
Supplementary Figure 1. MALDI-TOF spectra of lipid A extracted from PAO1 pME_{ptA} or PA14 pME_{ptA} cultured in the absence or presence of IPTG at 0.125 or 0.5 mM. The m/z values of peaks corresponding to phosphoethanolaminated lipid A forms are highlighted in yellow. Spectra are representative of three biological replicates giving similar results.



Supplementary Figure 2. Growth curves of (A) PAO1 or (B) PA14 carrying pME6032, pMEmcr-1 or pMEeptA in the presence of increasing IPTG concentrations (0-2 mM). Data represent the mean ($\pm$ standard deviation) of three independent experiments.

Supplementary Figure 3. Full-length alignment of selected EptA orthologs from *Acinetobacter baumannii* (SSI74383.1), *Enterobacter cloacae* (WP_059385803.1), *Escherichia coli* (OWW57050.1), *Helicobacter pylori* (WP_189394748.1), *Klebsiella pneumoniae* (CCI78008.1), *Neisseria meningitidis* (AKM91408.1), *Pseudomonas aeruginosa* (WP_003113468.1), *Salmonella enterica* (QNL54535.1), *Shigella flexneri* (QLG55136.1), *Vibrio cholerae* (TYC39410.1). The MCR-1 protein has been included as control (WP_049589868.1). The highly conserved catalytic residue mutated in the EptA^{T278A} variant and the 22 residues deleted in the EptA^{ΔC-ter} variant are highlighted with a black box and a red box, respectively.

<i>Helicobacter pylori</i>		170	MAISLFLHFLKPLSLCLQAGLIVSLIFGVLVHFPLFAVYVKE---SNQVSFIAPMVWVLCVINGALFLALGLISASLRRSRSAIVFSNLNSVAFYFISAYKVLWLSMNGVNLNTNTHIELLGFSLVKLFLVFIWFGVLPQWVYIKPLKISSKKAPFL-----AIALVLFIFIASALANA
MOB1		176	WQHTSVMYRSVSPFLVASAVVLTATAILTFDKISQTYPIA---SSYQVYLPLVILLINGAVTSYFTDVTGVTDTNLQALQDQAEKDKLNAAFITMIITGLGVLPSSLVAFKVDY-PTNGKGLHRRGLIVASLALTLPVVAES
<i>Vibrio cholerae</i>		174	--HPTF--NITP--FSYQLVFLLAAYFALPLNLPVQAQAHFNQSTSDAGSALPTPLFLFAHFFQFQ--FSHPVLVPEFAVLLSALSISAFAGVGVQVQDQNLVLLVLTORGEAGSYLTSVFNKGLGELGVPALALLFTPTPEESALRELKUGLSNLSVSVQVGTAGLVY
<i>Aeromonas aeruginosa</i>		161	-----HFLIATLNLGAILNFGVHQWHTLTPYFGKALLFVATLVLVTGYVYANLQI---LMIKATKATFAILLIFLGGFSSTYFHTIGVTSIQDQNNQWQTDNSEVDLISLRFVLMTFFVPLPFLITLTKNQKQ-ENVSLLLKKVLSVASFAVGVLLFTY
<i>Enterobacteriaceae</i>		170	PSKAR--AVAPPELL---TLVLSLAPLFLNLPQLLTAIPG-GWGLGPHLGLAGLIVGLVYANFALNTL---LAFPHKLPALPTLLAAGATFHTIGVTSIQDQNNQWQTDNSEVDLISLRFVLMTFFVPLPFLITLTKNQKQ-ENVSLLLKKVLSVASFAVGVLLFTY
<i>Neisseria meningitidis</i>		174	--NIXP--NLKPLGSSALFSLYSLSLVLYNVAFFAKVHEHFGHTGADFLVTPWVPLFGLSNVHFV---IALPFRHVLPLIPLVITSAASVQYEFNFNLSNLNMLQITTAESARLTVQWLVICLGVLPALAYTAKVYK--RWYKEFLTRVLAAVSLCAGLAAVQY
<i>Moraxella pneumoniae</i>		172	--HML--NLKPLQCDIKFTLGCALFF-TLNGLFTQSKAIIAPQALHDFEAFASPLVLCFGLNIVFSL---LNPFRKPLPLITVTHGCAATYFTYGAVIDQNMIVAFVETHSQEAALTVPQNLMTVLGVPSPVSLATLTHT--GWMYALLTRVAALLGALLVIAVAFY
<i>Salmonella enterica</i>		176	MSLLP--LRPVPVSGQIAMLILFSEYIATVCLNLAFYQVFPPLVMSLHMLVLSHPITVASVMSVLTLL--ASFLLKRLRLSLFLSLSASAQYFIMFGVIVDSRNTNITLDTTAAESFALLSGENITAVLGLSGVLAATFAWKIRPATPLRSGLYRLASVLSILLVIAAFY
<i>Escherichia coli</i>		176	HLKRL--LKRPVSGQIAMLILFSEYIATVCLNLAFYQVFPPLVMSLHMLVLSHPITVASVMSVLTLL--SSFLKRLRLSLFLSLSASAQYFIMFGVIVDSRNTNITLDTTAAESFALLSGENITAVLGLSGVLAATFAWKIRPATPLRSGLYRLASVLSILLVIAAFY
<i>Shigella flexneri</i>		176	HLKRL--LKRPVSGQIAMLILFSEYIATVCLNLAFYQVFPPLVMSLHMLVLSHPITVASVMSVLTLL--SSFLKRLRLSLFLSLSASAQYFIMFGVIVDSRNTNITLDTTAAESFALLSGENITAVLGLSGVLAATFAWKIRPATPLRSGLYRLASVLSILLVIAAFY
<i>Helicobacter pylori</i>		328	KMLWFDKHAFTIGFLTPFAYSNAFRVSLKFFAPT---IKPLPLSPNHSNSCWLVIGESAKNHVALGVQNPHTPLRSKLRLADNELTLNATSCATYTTASLDCILDSFKH-----NAYEMHPYTLTKAGIKVPHVWSANDGENKMYTSYKIVELIQK--
MOB1		351	SHYASFFRHKPLSYNIPNTYVSGLASIEYKASAPIIDIIVHAKDAVQAT---KPDNRKPLRVFWGETARADHSFNGYEDTFFQLAK-I-DGVTFNSVTSCEHTSTAVSVCNFSYLGAEYVDVDTAKYQENVLDTLDRGVSTLWRDINSDSIGVMDKLPKAPQADYSAT
<i>Vibrio cholerae</i>		347	QWYSVGNRMSLKKMLTPTLHLYSGFLGVRVTEPNVQE--YGOQAQHPVAVQKQKPTLVFVLGETARVQVQVLYGYPDTNAYTA--P-FQPIFFQVASCETATAVSVCNFSHNNRQNFQRADQNVLDLQAGTSLLMKENDSGDINAKNIPLEKARDNR--
<i>Aeromonas aeruginosa</i>		331	YDAATFREHNDLKNISQNTSSLSHSYHKKAPKKNLPLVI--YGOQAQHPVAVQKQKPTLVFVLGETARVQVQVLYGYPDTNAYTA--P-FQPIFFQVASCETATAVSVCNFSHNNRQNFQRADQNVLDLQAGTSLLMKENDSGDINAKNIPLEKARDNR--
<i>Neisseria meningitidis</i>		342	QGLSFLFRNNKELRLQVTPSNLIGAAIGYAKGAQAASQPLRP--IAVADARBAAL--HGGHARKSLTVLVGESARAQNLNGYARETNPLKA-E-EGLTFNSVNSHCEHTSTAVSVCNFSHNGRADYSADARTQEGLLDLVQORAGLRLVLRDQNSGCKGTCDVAFQDLESKD--
<i>Enterobacteriaceae</i>		344	QVYASFERNKSTHLLVPSNIFTGAGSKYDVK--KSNIPTQI--LDVAVQHR--PAGSLRRFVLVWGETTAAHGLNGVSTQITLLAAG-DEIVNEPQVSCETSTAVSVCNFSHNGRADYSADARTQEGLLDLVQORAGLRLVLRDQNSGCKGTCDVAFQDLESKD--
<i>Moraxella pneumoniae</i>		343	KDYASFERNKSTHLLVPSNIFTGAGSKYDVK--KSNIPTQI--LDVAVQHR--PAGSLRRFVLVWGETTAAHGLNGVSTQITLLAAG-DEIVNEPQVSCETSTAVSVCNFSHNGRADYSADARTQEGLLDLVQORAGLRLVLRDQNSGCKGTCDVAFQDLESKD--
<i>Salmonella enterica</i>		346	KDYASFERNKSTHLLVPSNIFTGAGSKYDVK--KSNIPTQI--LDVAVQHR--PAGSLRRFVLVWGETTAAHGLNGVSTQITLLAAG-DEIVNEPQVSCETSTAVSVCNFSHNGRADYSADARTQEGLLDLVQORAGLRLVLRDQNSGCKGTCDVAFQDLESKD--
<i>Escherichia coli</i>		346	KDYASFERNKSTHLLVPSNIFTGAGSKYDVK--KSNIPTQI--LDVAVQHR--PAGSLRRFVLVWGETTAAHGLNGVSTQITLLAAG-DEIVNEPQVSCETSTAVSVCNFSHNGRADYSADARTQEGLLDLVQORAGLRLVLRDQNSGCKGTCDVAFQDLESKD--
<i>Shigella flexneri</i>		346	KDYASFERNKSTHLLVPSNIFTGAGSKYDVK--KSNIPTQI--LDVAVQHR--PAGSLRRFVLVWGETTAAHGLNGVSTQITLLAAG-DEIVNEPQVSCETSTAVSVCNFSHNGRADYSADARTQEGLLDLVQORAGLRLVLRDQNSGCKGTCDVAFQDLESKD--
<i>Helicobacter pylori</i>		492	--CPNCE--ATAPOVESLLYVLPDLLKEHSIE---NVLILHLAGSHGPWDRVLPNFRVFKPYCSSADLSGCSKESLINAVDNITFYVDYLDLQIISMLEKA--KQALNITVLSOHGESLGEAPYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
MOB1		519	NIATCNTIPMECRDVGHLVLDQFVAANNGK---DMLILHLQIGHGPAYFKRYDEKFAKFTPYVCEGNELAKCEHQSILINAVDNALLATDDFIAQSTQNLQTHSNAYDVSNILVSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Vibrio cholerae</i>		518	--EGICD--GDTCYDIANLENDQETATQGN---RMIFNHFIGSHGTYFKRYDEKFAKFTPYVCEGNELAKCEHQSILINAVDNALLATDDFIAQSTQNLQTHSNAYDVSNILVSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Aeromonas aeruginosa</i>		505	--KRWCK--DGEYDIDILDLQKQYLSIAKDDDRPLRLVHVGSHGPAYVYKAPAEVQPEKPTCTNAGISQTELLNSYDNTIVYDHLVLSQMTLKEI--SKYQTGLVYSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Neisseria meningitidis</i>		513	--PALCA--GGECHOELLRLQKQYLSIAKDDDRPLRLVHVGSHGPAYVYKAPAEVQPEKPTCTNAGISQTELLNSYDNTIVYDHLVLSQMTLKEI--SKYQTGLVYSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Enterobacteriaceae</i>		515	--PEYCR--NGECHOELLRLQKQYLSIAKDDDRPLRLVHVGSHGPAYVYKAPAEVQPEKPTCTNAGISQTELLNSYDNTIVYDHLVLSQMTLKEI--SKYQTGLVYSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Moraxella pneumoniae</i>		515	--DQCEK--DQCECHOELLRLQKQYLSIAKDDDRPLRLVHVGSHGPAYVYKAPAEVQPEKPTCTNAGISQTELLNSYDNTIVYDHLVLSQMTLKEI--SKYQTGLVYSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Salmonella enterica</i>		518	--TGQCI--DQCECHOELLRLQKQYLSIAKDDDRPLRLVHVGSHGPAYVYKAPAEVQPEKPTCTNAGISQTELLNSYDNTIVYDHLVLSQMTLKEI--SKYQTGLVYSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Escherichia coli</i>		518	--PQCI--DQCECHOELLRLQKQYLSIAKDDDRPLRLVHVGSHGPAYVYKAPAEVQPEKPTCTNAGISQTELLNSYDNTIVYDHLVLSQMTLKEI--SKYQTGLVYSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Shigella flexneri</i>		518	--PQCI--DQCECHOELLRLQKQYLSIAKDDDRPLRLVHVGSHGPAYVYKAPAEVQPEKPTCTNAGISQTELLNSYDNTIVYDHLVLSQMTLKEI--SKYQTGLVYSDHGESLGENGYVHGPDKISTAPKEQVEPTIVVAINEPEKFKHSTVQ-----TOTPINQWTF
<i>Helicobacter pylori</i>		521	HSLLGVFLDFKPSAVYRPSLDLLKHKE-----
MOB1		541	PTLLKLFV--TADVORTAFT-----
<i>Vibrio cholerae</i>		547	HSLLGVMDV--ST-KAYQANLDLFAKRTSQS-----
<i>Aeromonas aeruginosa</i>		533	PSLSLDDV--RT-QVNPQLDHLHSAHN-----
<i>Neisseria meningitidis</i>		567	HSPLGLLEV--RT-GAYDGLDLFAPCAPVYLQ[LKADGRALVSEASGQEPPLAS
<i>Enterobacteriaceae</i>		544	STVLGLMDI--SHSQTYRKEVDILAACRRPR-----
<i>Moraxella pneumoniae</i>		541	STVLGMWDV--KS-TVYQQQLDLINACRQ-----
<i>Salmonella enterica</i>		546	STVLGLLVV--ST-REYQAADILTPCREAG-----
<i>Escherichia coli</i>		547	STVLGLTVV--QT-TYQAADILTPCRELSE-----
<i>Shigella flexneri</i>		547	STVLGLTVV--ET-KYQAADILTPCREVSE-----
		547	STVLGLTVV--ET-KYQAADILTPCREVSE-----



Supplementary Figure 4. MALDI-TOF spectra of lipid A extracted from PAO1 or PA14 cells expressing EptA variants mutated in a catalytic residue (EptA^{T278A}) or deleted of 22 amino acids at the C-terminus (EptA^{ΔC-ter}) cultured in the presence of 0.5 mM IPTG. The m/z values of peaks corresponding to phosphoethanolaminated lipid A forms are highlighted in yellow. Spectra are representative of three biological replicates giving similar results.

1.2. Supplementary Table

Supplementary Table 1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Genotype and/or relevant characteristics	Reference or source
<i>P. aeruginosa</i>		
PAO1 (ATCC15692)	Reference isolate	American type culture collection
PA14	Reference isolate	Rahme et al., 1995
PAO1 <i>PrpsA::arn</i>	PAO1 derivative in which the promoter of the <i>arn</i> operon is replaced by the promoter of the housekeeping gene <i>rpsA</i>	Lo Sciuto et al., 2020
PA14 <i>PrpsA::arn</i>	PA14 derivative in which the promoter of the <i>arn</i> operon is replaced by the promoter of the housekeeping gene <i>rpsA</i>	Lo Sciuto et al., 2020
<i>E. coli</i>		
DH5 α F'	<i>recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 Δ(lacZYA-argF)U169[ϕ80 <i>dlacZ</i>ΔM15], NaI^R</i>	Liss, 1987
Plasmid		
pHNSHP45	<i>mcr-1</i> carrying plasmid	Liu et al., 2016
pBluescript II KS (pBS)	Sequencing vector; ColE1 replicon; Ap ^R	Stratagene
pBSeptA	pBS containing the coding sequence of the <i>eptA</i> gene from <i>P. aeruginosa</i> PAO1 (PA1972)	This study
pBSmcr-1	pBS containing the coding sequence of the <i>mcr-1</i> gene from plasmid pHNSHP45	This study
pBSeptA ^{T278A}	pBS containing the <i>eptA</i> coding sequence with a point mutation in the codon 278 leading to a T278A substitution	This study
pBSeptA ^{ΔC-ter}	pBS containing a truncated <i>eptA</i> allele encoding for an EptA variant lacking the last 22 amino acids	This study
pME6032	IPTG-inducible expression vector, <i>lacI</i> ^Q , Tc ^R .	Heeb and Haas, 2001
pMEeptA	pME6032 containing the <i>eptA</i> (PA1972) coding sequence from pBSeptA cloned downstream of the IPTG-inducible promoter	This study
pMEmcr-1	pME6032 containing the <i>mcr-1</i> coding sequence from pBSmcr-1 cloned downstream of the IPTG-inducible promoter	This study
pMEeptA ^{T278A}	pME6032 containing the <i>eptA</i> ^{T278A} allele from pBSeptA ^{T278A} cloned downstream of the IPTG-inducible promoter	This study
pMEeptA ^{ΔC-ter}	pME6032 containing the <i>eptA</i> ^{ΔC-ter} allele from pBSeptA ^{ΔC-ter} cloned downstream of the IPTG-inducible promoter	This study

Supplementary Table 2. Primers used in this study.

Primer name	Sequence (5'-3') ¹	Restriction sites ²	Application
<i>mcr-1</i> _pME6032_FW	cgg <u>aattc</u> ATGATGCAGCATACTTCTGTG	EcoRI	Generation of pBS <i>mcr-1</i>
<i>mcr-1</i> _pME6032_RV	ccc <u>tcgag</u> TCAGCGGATGAATGCGGTG	XhoI	
<i>eptA</i> _pME6032_FW	cgg <u>aattc</u> CATGTCGAAAGCCCCGCGC	EcoRI	Generation of pBS <i>SeptA</i>
<i>eptA</i> _pME6032_RV	ccc <u>tcgag</u> GTATCAGGAAGCCGGCGG	XhoI	
<i>eptA</i> _T278A_FW	CGGTACCGAGgCCGCGGTGTC		Generation of pBS <i>SeptA</i> ^{T278A}
<i>eptA</i> _T278A_RV	CAGGAGTGCACGTTGGAGAAGTTGATC		
<i>eptA</i> _Δ22aa_RV ³	ccc <u>tcgag</u> tcaCGCCTGCAGGACCTCGGG	XhoI	Generation of pBS <i>SeptA</i> ^{ΔC-ter}
<i>eptA</i> _RT_FW ⁴	TGCCCTGCATGTTCTCCAAC		qRT-PCR
<i>eptA</i> _RT_RV ⁴	GATCCTTGCTCTCGCTCAGG		
<i>rpoD</i> _RT_FW	GGGCGAAGAAGGAAATGGTC		
<i>rpoD</i> _RT_RV	CAGGTGGCGTAGGTGGAGAA		
M13_FW	GTTTTCCTCCAGTCACGAC		DNA sequencing from pBS
M13_RV	AACAGCTATGACCATG		

¹ Lowercase letters indicate the region of the primer that does not anneal to the template.

² The restriction site used for cloning is underlined in the primer sequence.

³ This primer was paired with *eptA*_pME6032_FW to amplify the truncated *eptA*^{ΔC-ter} variant.

⁴ Primers described and used in Nowicki et al., 2015.

Additional references (not included in the main text)

Liss, L. (1987). New M13 host: DH5 F' competent cells. *Focus* 9, 13.

Rahme, L.G., Stevens, E.J., Wolfort, S.F., Shao, J., Tompkins, R.G., Ausubel, F.M. (1995). Common virulence factors for bacterial pathogenicity in plants and animals. *Science* 268, 1899-1902.