

Supplementary Information

A novel siderophore system is essential for the growth of *Pseudomonas aeruginosa* in airway mucus

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Table S1. Bacterial strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	Ref. or source
<i>P. aeruginosa</i> strains		
PAO1	Wild-type PAO1, ATCC	Lab collection
$\Delta PA4834$	PAO1, <i>PA4834</i> deleted	This study
Δpvd	PAO1, <i>PA2396</i> and <i>PA2397</i> deleted	This study
Δpch	PAO1, <i>PA4224</i> and <i>PA4225</i> deleted	This study
$\Delta pvd\Delta pch$	PAO1, <i>PA2396</i> , <i>PA2397</i> , <i>PA4224</i> and <i>PA4225</i> deleted	This study
$\Delta PA4834\Delta pvd$	PAO1, <i>PA4834</i> , <i>PA2396</i> and <i>PA2397</i> deleted	This study
$\Delta PA4834\Delta pch$	PAO1, <i>PA4834</i> , <i>PA4224</i> and <i>PA4225</i> deleted	This study
$\Delta PA4834\Delta pvd\Delta pch$	PAO1, <i>PA4834</i> , <i>PA2396</i> , <i>PA2397</i> , <i>PA4224</i> and <i>PA4225</i> deleted	This study
$\Delta PA4834::Tn$	<i>PW9128</i> , <i>PA4834::ISlacZ/hah</i> , UW strain	60
$\Delta PA4834/pJN105$	$\Delta PA4834$, empty vector control	This study
$\Delta PA4834/pJN105::PA4834$	$\Delta PA4834$ complementation strain	This study
<i>E. coli</i> strains		
SM10/ λ pir	Km^r <i>thi-1 thr leu tonA lacY supE recA::RP4-2-Tc::Mu pir⁺</i> , for conjugal transfer	61
Other bacterial species		
<i>Staphylococcus aureus</i>		Lab collection
<i>Bacillus cereus</i>		Lab collection
<i>Escherichia coli</i>	DH5 α	Lab collection
<i>Vibrio cholerae</i>	N16961	Lab collection
<i>Salmonella enterica</i> serovar Typhimurium	LT2	Lab collection
<i>Listeria monocytogenes</i>		Lab collection
Plasmids		
pCVD442	<i>sacB</i> suicide vector from plasmid pUM24	61
pJN105	pJN105 <i>araC</i> -PBAD cassette cloned in pBBR1MCS-5, Gm^r	57
pJN105:: <i>PA4834</i>	pJN105 inducible vector with fragment of <i>PA4834</i>	This study

Table S2. Primers used in this study

Gene name	Direction	Primer sequence (5'-3') ^a	Restriction sites
Mutant construction			
<i>PA4834</i> left	Forward	ATATAGAGCT <u>CGCCGAGCGCCAGGAATACCTG</u>	SacI
<i>PA4834</i> left	Reverse	TAAATGGAT <u>CCCAGCAGGGTACCGATGGCGC</u>	BamHI
<i>PA4834</i> right	Forward	CCCTTGGAT <u>CCGGCATCGTCTTCAACGCCGG</u>	BamHI
<i>PA4834</i> right	Reverse	AAGGTGAGCTCAGCCTTTCTGCCTGGTCACG	SacI
<i>pvd</i> left	Forward	ACACGGAGCTCCCTTCCCGTTCGACGAAGGTA	SacI
<i>pvd</i> left	Reverse	CTTCCGGATCCTCACCCGACGACACCATCCTC	BamHI
<i>pvd</i> right	Forward	AGAGCGGATCCAATGCCTGGCTCGAAGAGCGAC	BamHI
<i>pvd</i> right	Reverse	CGATTGAGCTCACTCCTGGAGTGCCTGCC	SacI
<i>pch</i> left	Forward	ACACGGAGCTCAATGGCAAGGTCGATCGTCGC	SacI
<i>Pch</i> left	Reverse	CTTCCGGATCCAGGTGTTCCAGCAGGGCCTC	BamHI
<i>pch</i> right	Forward	AGAGCGGATCCAACCAGCGCAGCCTGGAGGT	BamHI
<i>pch</i> right	Reverse	CGATTGAGCTCAGCGTGCCTGATCAGTTGGT	SacI
complementation			
<i>PA4834</i> complementation (cloning to pET21b)	Forward	CTTACCATATGGTGCTCGACCTGCTGAAGAGC	NdeI
	Reverse	AAATTAAGCTTGCCCTTCTTCGTCCGCGACAG	HindIII
<i>PA4834</i> complementation (cloning to pJN105)	Forward	ACTCTGAATTCAATAATTTTGTAACTTTAAGAAGGAG	EcoRI
	Reverse	ATTGGTCTAGATCAGTGGTGGTGGTGGT	XbaI
Quantitative RT-PCR			
<i>PA4834</i>	Forward	GACCTGCTGAAGAGCGGGGT	
	Reverse	GCTTCCTTGACCAGGGCGTT	
<i>PA4835</i>	Forward	TACGTGCACAGTCCGTTCTT	
	Reverse	GATAGGTCCCTCCGGGTAGA	
<i>PA4836</i>	Forward	CTGTTCAACTATCCGGTGGAG	
	Reverse	TTCTCCAGGATCAGGGTGTC	
<i>PA4837</i>	Forward	ACCTTGACAGCGCTCGTATC	
	Reverse	AAGCCACGGACGTTGTACTC	
<i>rpoD</i> (PA0576)	Forward	AAGGCCCTGAAGAAGCACGG	
	Reverse	GATCGGCATGAACAGCTCGG	

^a Restriction enzyme recognition sequences are underlined.

Table S3. BLASTN search of PA4834, PA4835, PA4836 and PA4837 gene sequences

Genes	PA4834	PA4835	PA4836	PA4837
Predicted function	EamA-like transporter family protein	Conserved uncharacterized	Nicotianamine synthase	TonB-dependent siderophore receptor
Bacterial species that contain genes homologous to PA4834, PA4835, PA4836 or PA4837				
<i>P. aeruginosa</i> PA01	855 / 855 (100%)	1302 / 1302 (100%)	792 / 792 (100%)	2127 / 2127 (100%)
<i>P. aeruginosa</i> DK2	855 / 855 (100%)	1296 / 1302 (99%)	787 / 792 (99%)	2123 / 2127 (99%)
<i>P. aeruginosa</i> LESB58	853 / 855 (99%)	1295 / 1302 (99%)	784 / 792 (99%)	2114 / 2127 (99%)
<i>P. aeruginosa</i> M18	853 / 855 (99%)	1296 / 1302 (99%)	786 / 792 (99%)	2120 / 2127 (99%)
<i>P. aeruginosa</i> RP73	853 / 855 (99%)	1297 / 1302 (99%)	786 / 792 (99%)	2118 / 2127 (99%)
<i>P. aeruginosa</i> PA96	854 / 855 (99%)	1299 / 1302 (99%)	785 / 792 (99%)	2115 / 2127 (99%)
<i>P. aeruginosa</i> UCBPP-PA14	846 / 854 (99%)	1286 / 1302 (99%)	783 / 792 (99%)	2108 / 2127 (99%)
<i>P. aeruginosa</i> PA7	788 / 855 (92%)	1188 / 1302 (91%)	757 / 792 (96%)	2001 / 2127 (94%)
<i>P. protegens</i> Cab57	—	—	—	1436 / 2007 (72%)
<i>P. stutzeri</i> A1501	—	—	—	1465 / 2062 (71%)
<i>P. mendocina</i> ymp	—	—	—	1478 / 2094 (71%)
<i>P. mandelii</i> JR-1	—	—	—	1432 / 2017 (71%)
<i>Burkholderia</i> sp. 383	577 / 828 (70%)	—	—	—
<i>Serratia</i> sp. FS14	530 / 782 (68%)	255 / 373 (68%)	—	—
<i>Serratia marcescens</i> WW4	514 / 759 (68%)	—	—	—
<i>Arsenophonus nasoniae</i>	487 / 726 (67%)	—	—	—
<i>Vibrio nigripulchritudo</i> str. SFn1	250 / 385 (65%)	—	—	—
<i>Actinosynnema mirum</i> DSM 43827	—	207 / 285 (73%)	—	—
<i>Bordetella bronchiseptica</i> 253	—	—	38 / 43 (88%)	—
<i>Bordetella bronchiseptica</i> MO149	—	—	38 / 43 (88%)	—
<i>Azotobacter vinelandii</i> CA6	—	—	—	1480 / 2023 (73%)
<i>Azotobacter vinelandii</i> CA	—	—	—	1480 / 2023 (73%)

Table S4. Antibiotic sensitivity test

Antibiotics	Inhibition zone (mm)	
	PA01	$\Delta PA4834$
Amikacin	24	26
Ceftazidime	27	29
Ciprofloxacin	34	33
Gentamicin	19	21
Tobramycin	26	26
Cefoperazone-sulbactam	27	27
Piperacillin	28	28
Imipenem	24	26
Piperacillin-tazobactam	29	30
Aztreonam	26	27
Meropenem	31	31
Cefepime	30	31
Colistin	13	13