Bacterial strains and growth conditions

Bacterial strains are described in the table below. Strain JFC207 and JFC234 were constructed using P1 transduction standard procedures (1). The *E. coli* CC118λpir strain was used to propagate pKNG101 and the derivative plasmids, while TGI and TOP10F' strains were used for other plasmid manipulations.

E. coli cultures were grown in LB or TSB medium at 37°C with shaking (200 rpm). When required, media were supplemented with ampicillin (50 or 200 µg/ml), kanamycin (25 or 50 µg/ml), streptomycin (30 µg/ml), gentamycin (15 µg/ml), L-arabinose (0.2 %) or D-glucose (0.4 %). To prepare *E. coli* periplasmic extracts, bacteria were grown in M63 minimal medium.

P. aeruginosa cultures were grown in LB or TSB medium at 37°C with shaking (130 rpm). When required, media were supplemented with streptomycin (2 mg/ml) or gentamycin (150 µg/ml). *P. aeruginosa* transconjugants were screened on *Pseudomonas* isolation agar (PIA, Difco laboratories).

Plasmid constructions

The plasmids and primers used in the present study are listed in the table below. The PaDsbA1, PaDsbA2, PaDsbB1 and PaDsbB2 expression vectors were constructed as follows: the coding DNA sequences encoding *PadsbA1*, *PadsbA2*, *PadsbB1* and *PadsbB2* were amplified from the chromosome of *P. aeruginosa* PA14 strain using the primers listed in the table below and inserted into the pET23a vector. PaDsbA1 and PaDsbA2 were cloned with and without signal sequence. Amplification reactions were run in 50 ml reaction volumes containing Expand High Fidelity buffer 1X (kit Expand High Fidelity PCR System, Roche), 10 mM $MgCl_2$, 1 µM of each primer (Fw and Rv), 0.2 mM of each dNTP, 0.36 % DMSO or 60 mM betaine and 0.05 U/µl of Hifi polymerase (Roche). 1 µl of genomic DNA (*P.*

aeruginosa PA14 - 50 ng/µl - obtained using the Pure link Genomic DNA Minikit, Invitrogen) was used as matrix. Hybridation was performed at 62°C for 30 sec, during 20 cycles. Sequences were checked by DNA sequencing (Beckman Coulter Genomics).

For kinetic experiments with membrane or total cell extracts, genes were subcloned from pET23a to pBAD33. The *PadsbA1*, *PadsbA2*, *PadsbB1* and *PadsbB2* genes were amplified from the pET23a expression vector using the primers listed in the table below. The ribosome binding site from pET23a was included in the amplified sequence. Amplification was done as previously described, using 480 to 900 ng of the various pET23a vectors as template. DNA samples were sent to Beckman Coulter Genomics (Essex, United Kingdom) to be sequenced on ABI* 3730XL.

For *P. aeruginosa*, plasmid preparations were performed using the QIAPrep spin kit (Qiagen). Recombinant plasmids were introduced in *P. aeruginosa* using the conjugative properties of pRK2013.

The expression plasmid for PaDsbA2$_{CCSS}$ was constructed by site-directed mutagenesis using the QuickChange Mutagenesis Protocol (Stratagene). Plasmid pETdsbA2-SS (without signal sequence) was used as a template. The expression plasmid for PaDsbA2$_{CCSS}$ was generated by replacing the codons for cysteines 111 and 157 by serine codons using primers PadsbA2C111S Fw and PadsbA2C111S Rv first and then primers PadsbA2C157S Fw and PadsbA2C157S Rv, yielding plasmid pETdsbA2-SS$_{CCSS}$.

Construction of PadsbA1, *PadsbA2*, *PadsbB1*, *PadsbB2* and *PadsbB1B2 mutants*

Unmarked, in-frame deletions of *PadsbA1*, *PadsbA2, PadsbB1* and *PadsbB2* were constructed in *P. aeruginosa* PA14 via allelic exchange as described in Ball et al. (2012) (2). 500 bp upstream (-500) and downstream (+500) regions of the target genes were PCR amplified and tandemly cloned in the pKNG101 using the -500Up/+500Dw primer pairs. The

resulting constructs (pKNΔ*PadsbA1*, pKNΔ*PadsbA2,* pKNΔ*PadsbB1* and pKNΔ*PadsbB2*) were transferred by conjugation in the wild type *P. aeruginosa* PA14 strain in order to generate the *PadsbA1*, *PadsbA2*, *PadsbB1* and *PadsbB2* mutants. The double mutant *PadsbB1B2* was constructed by deleting the *PadsbB2* gene in the P*adsbB1* mutant.

Protein expression and purification

Plasmids pET23a::dsbA1-SS and pET23a::dsbA2-SS_{CCSS} were transformed into BL21 (DE3) competent cells, yielding strains IA14 and IA38, respectively. Plasmid pET23a::dsbA2-SS was transformed in Rosetta Gami competent cells, yielding strain IA20. During overnight cultures in LB medium containing 200 µg/L of ampicillin at 37°C without shaking, cells reached an OD_{600nm} between 0.5 and 0.8. Cells were then cultured aerobically (200 rpm shaking) at 37°C. After 30 min, protein expression was induced by adding 1 mM IPTG. Cells were harvested by centrifugation (Beckman Coulter JA-10 rotor, 2,800 × *g*, 4°C, 20 min) after 4h of induction. Cells were resuspended in 10 mL buffer A containing 50 mM NaPi pH 8.0 and 300 mM NaCl and disrupted by two passages through a French press (1500 psi). Cells lysates were then centrifuged during 40 min (Beckman Coulter JA-20 rotor, 23,600 × *g*, 4°C). Supernatants were recovered and diluted about 5 times in buffer A and filtered with a Minisart High-Flow filter (Sartorius, pores of 0.2 µm diameter). Diluted supernatants were loaded on a HisPur Ni-NTA Chromatography Cartridge (1 ml, Thermo Scientific) at 1 ml/min. After washing the column, proteins were eluted using an imidazole gradient (0 to 180 mM in buffer A). The fractions containing the proteins were concentrated using a Vivaspin-15 device (Sartorius) and desalted on a PD-10 column (GE healthcare) equilibrated with buffer B (50 mM NaPi pH 8.0 and 150 mM NaCl).

After the affinity chromatography, the proteins were loaded onto a Q-Sepharose column (GE healthcare) equilibrated with buffer C (20 mM Tris pH 8.0). The column was

washed with buffer C and PaDsbA2 was eluted with a 0 mM - 400 mM NaCl gradient in buffer C. The fractions containing the proteins were pooled, concentrated using a Vivaspin-15 device and desalted on a PD-10 column equilibrated with buffer B.

PaDsbA1 and PaDsbA2 were then loaded on a Superdex 200 gel filtration column equilibrated with buffer D (25 mM Hepes, pH 7.7, 100 mM NaCl) at a flow rate of 1 ml/min. The fractions containing the proteins were concentrated and the pure proteins stored in buffer D. Optical spectra were recorded with a Varian Cary 50 Bio UV-spectrophotometer.

The PaDsbA2 mutant protein ($PaDsbA2_{CCSS}$) was purified by two successive passages through a nickel column HisPur Ni-NTA Chromatography Cartridge (Thermo Scientific), concentrated (Vivaspin-15) and desalted on a PD-10 column equilibrated with buffer A.

To express PaDsbB1 and PaDsbB2, pET23a::dsbB1 and pET23a::dsbB2 were transformed in *E. coli* BL21C43 competent cells, yielding strains IA17 and IA18, respectively. Cells were cultured aerobically in LB medium containing 200 µg/L of ampicillin at 37°C until they reached an OD_{600nm} about 0.7. Expression of the proteins was induced by adding IPTG (500 µM for IA17 and IA18; 20 µM for IA53). Cells were harvested by centrifugation (Beckman Coulter JA-10 rotor, 2,800 × *g*, 20 min, 4°C) after 4h for IA17 and IA53 and 16h for IA18. Cells were resuspended in 10 mL buffer A containing 50 mM NaPi pH 8.0 and 300 mM NaCl and disrupted by two passages though a French press (1500 psi). The suspensions were then ultracentrifuged (Beckman Coulter 55.2 Ti rotor, 179,300 × *g,* 1h, 4°C). After centrifugation, the pellets were homogenized using a potter in 6.4 mL of buffer A supplemented with a cocktail of protease inhibitors (Roche (complete, EDTA-free)). Membrane proteins were solubilized using 2% N-dodecyl-β-D-maltoside (DDM) during 1h30 at 4°C on a roller. Proteins were recovered in the supernatant fraction after 1h ultracentrifugation (Beckman Coulter 55.2 Ti rotor, 179,300 × *g*, 4°C). Supernatants were diluted to a final volume of 50 mL, in buffer A containing 0.02% DDM and were loaded on

HisPur Ni-NTA Chromatography Cartridge (1 ml, Thermo Scientific) at 1 ml/min. After washing the column, proteins were eluted using an imidazole gradient (0 to 180 mM in buffer A). The fractions containing the proteins were concentrated using a Vivaspin-15 device (Sartorius) and desalted on a PD-10 column (GE healthcare) equilibrated with buffer E (50 mM NaPi pH 8.0, 150 mM NaCl and 0.1 % DDM).

Strains	**Genotype and characteristics**	**References**
***E. coli* strains**		
MC1000	F$^-$ λ^- *araD*139 Δ*(ara-leu)*7697 Δ*(lacIY)*74 *galU galK rpsL*	(3)
XL-1-Blue	*endA1 gyrA96*(nal^R) *thi-1 recA1 relA1 lac glnV44* F'[::Tn10 *proAB*$^+$ *lacI*^{q} *Δ(lacZ)M15] hsdR17*(r_K^- m_K^+)	Stratagene
BL21 (DE3)	F$^-$ *ompT gal dcm lon hsdS$_B$*(r_B^- m_B^-) λ(DE3 [*lacI lacUV5-T7 gene 1 ind1 sam7 nin5*])	(4)
BL21C43 (DE3)	F$^-$ *ompT gal dcm lon hsdS$_B$*(r_B^- m_B^-) λ(DE3 [*lacI lacUV5-T7 gene 1 ind1 sam7 nin5*]) and two uncharacterised mutations	(5)
Rosetta-gami (DE3)	*Δ(ara-leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL* (DE3) F'[*lac*$^+$ *lacI*^{q} *pro*] *gor522*::Tn10 *trxB* pRARE (Cam^R, Kan^R, Str^R, Tet^R)	Stratagene
TG1	*supE, hsd*Δ*R, thi*Δ *(lac-proAB),* F' (*traD36, proAB+, lacIq, lacZ*Δ*M15*)	Laboratory collection
CC118 (*λpir*)	*(λpir) Δ (ara-leu), araD, ΔlacX74, galE, galK, phoA-20, thi-1, rpsE, rpoB, Arg* (Am), *recA1,* Rfr (*λpir*)	(6)
TOP10F'	F- *mcrA* Δ*(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL*(Str^R) *endA1* λ$^-$	Invitrogen
JFC234	MC1000 *dsbB*::kan	Laboratory collection
IA14	BL21 carrying pETdsbA1-SS	This study
IA20	Rosetta-gami carrying pETdsbA2-SS	This study
IA17	BL21C43 carrying pETdsbB1	This study
IA18	BL21C43 carrying pETdsbB2	This study
IA38	BL21 carrying pETdsbA2-SS$_{CCSS}$	This study
IA32	JFC234 carrying pBADdsbB1	This study
IA33	JFC234 carrying pBADdsbB2	This study
IA34	JFC234 carrying pBAD33	This study
***P. aeruginosa* strains**		
PA14	PA14 clinical isolate from human burn patient	(7)
PadsbA1	PA14 harboring a chromosomic deletion of *PadsbA1*	This study
PadsbA2	PA14 harboring a chromosomic deletion of *PadsbA2*	This study
PadsbB1	PA14 harboring a chromosomic deletion of *PadsbB1*	This study
PadsbB2	PA14 harboring a chromosomic deletion of *PadsbB2*	This study
PadsbA1A2	PA14 harboring a chromosomic deletion of *PadsbA1* & *PadsbA2*	This study
PadsbB1B2	PA14 harboring a chromosomic deletion of *PadsbB1* & *PadsbB2*	This study

Plasmids	**Characteristics**	**References**
pBAD33	L-arabinose inducible, Cmr, p15A origin	(8)
pET23a	T7 promoter, Ampr, f1 origin, C-terminal His6-tag	Novagen
pET*PadsbA1-SS*	pET23a expressing PaDsbA1 (without signal sequence)	This study
pET*PadsbA2-SS*	pET23a expressing PaDsbA2 (without signal sequence)	This study
pET*PadsbB1*	pET23a expressing PaDsbB1	This study
pET*PadsbB2*	pET23a expressing PaDsbB2	This study
pET*PadsbA2-SS*$_{CCSS}$	pET23a expressing PaDsbA2 (without signal sequence, cysteines 111 and 157 are replaced by serine)	This study

pBAD*PadsbB1*	pBAD33 expressing PaDsbB1	This study
pBAD*PadsbB2*	pBAD33 expressing PaDsbB2	This study
pCR2.1	TA cloning, lacZ, ColE1, f1 ori, ApR KmR	Invitrogen
pRK2013	Tra+, Mob+, ColE1, KmR	(9)
pKNG101	oriR6K, mobRK2, sacBR+, SmR (suicide vector)	(10)
pJN105	GmR ,araC-pBAD,(broad host range vector)	(11)
pKN*ΔPadsbA1*	500 bp upstream and 500 bp downstream PadsbA1 in pKNG101	This study
pKN*ΔPadsbA2*	500 bp upstream and 500 bp downstream PadsbA2 in pKNG101	This study
pKN*ΔPadsbB1*	500 bp upstream and 500 bp downstream PadsbB1 in pKNG101	This study
pKN*ΔPadsbB2*	500 bp upstream and 500 bp downstream PadsbB2 in pKNG101	This study
pJN*PadsbA1*	860 bp DNA fragment containing the PadsbA1gene cloned in pJN105	This study
pJN*PadsbB1*	620bp DNA fragment containing the PadsbB1gene cloned in pJN105	This study
pJN*PadsbB2*	617bp DNA fragment containing the PadsbB2 gene cloned in pJN105	This study

Primers	**Sequences**
Cloning	
PA14*PadsbA1* Fw (without SS)	TTACATATGGACGACTATACCGCCGGC
PA14*PadsbA1* Rv	TTACTCGAGCTTCTTGGCCGCTGCGCGC
PA14*PadsbA2* Fw (without SS)	TTACATATGGTAGAGCTTCTGGTGAAG
PA14*PadsbA2* Rv	TTACTCGAGGAGATCCTTGGCTAGCCAG
PA14*PadsbB1* Fw	TTACATATGAGCGCTCTCCTCAAGCC
PA14*PadsbB1* Rv	TTACTCGAGGGCGGTGCGGCGGCC
PA14*PadsbB2* Fw	TTACATATGCCCCTGGCCAGCCC
PA14*PadsbB2* Rv	TTACTCGAGGGCACGTCGGAGGAACTG
pET*Padsb* Fw	TTAGAGCTCTAATTTTGTTTAACTTTAAGAAGG
pET*PadsbB1* Rv	TTAGTCGACTCAGGCGGTGCGGCGGCCG
pET*PadsbB2* Rv	TTAGTCGACTCAGGCACGTCGGAGGAACTGC
pJN*PadsbA1* Fw	GTCACAGGAGGGGTTCACTC
pJN*PadsbA1* Rv	TACAATGCTGGAACCCGTGA
pJN*PadsbA2* Fw	ACATCCACAGCCTGATACGG
pJN*PadsbA2* Rv	CCAAAAGAGGGATGTTGGAA
pJN*PadsbB1* Fw	TAGCGAATTCCTGCAGTTAATCTTCGGGCAATCCTG
pJN*PadsbB1* Rv	TTAATGGTGATGGTGATGATGGGCGGTGCGGCGGCCGAA
pJN*PadsbB2* Fw	TAGCGAATTCCTGCAGGCATGATTCGGGATGTCTTG
pJN*PadsbB2* Rv	TTAATGGTGATGGTGATGATGGGCACGTCGGAGGAACTGCAC
Site directed mutagenesis	
PaDsbA2C111S Fw	CAGGCTCGCCTGGTGGAGAGCGCGGGGATCCAAGGCGGC
PaDsbA2C111S Rv	GCCGCCTTGGATCCCCGCGCTCTCCACCAGGCGAGCCTG
PaDsbA2C157S Fw	CAGGCTCGACTGGAGAAAAGTGCGAAAGACAACGAACTTATTG
PaDsbA2C157S Rv	CAATAAGTTCGTTGTCTTTCGCACTTTTCTCCAGTCGAGCCTG
Gene deletion	
PadsbA1-500Up	GCAGATGCATGACATCAAGGAC
PadsbA1-500Dw	CACCTTGCCTAACGCATCGTTCACTCCTA
PadsbA1+500Up	GAGTGAACGATGCGTTAGGCAAGGTGATTCG
PadsbA1+500Dw	AGATGCATCATCACTACCACCAG
PadsbA2-500Up	CGAAGTAGAGAAGATATCCAGGTTC
PadsbA2-500Dw	GCGACGCTAGAGGAGTCTCACGCTTGC
PadsbA2+500Up	GCGTGAGACTCCTCTAGCGTCGCGCC
PadsbA2+500Dw	AGCAGTACCAGTAGTACGCCAAC
PadsbB1-500Up	AAGGGGATCAACAGGATGC
PadsbB1-500Dw	CGGCGCTGTCAGGCGCTCAAGGCAGGGATTC
PadsbB1+500Up	CCTGCCTTGAGCGCCTGACAGCGCCGATATC
PadsbB1+500Dw	GTATTCCTGTACCGACTTGACGTAG
PadsbB2-500Up	AGGAAGAGGCTGAAGAAGTCCT
PadsbB2-500Dw	GTCAGGCACGTCGCATGGCGGATCCACAG
PadsbB2+500Up	GTGGATCCGCCATGCGACGTGCCTGAC

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