Table S1 Bacterial strains and plasmids used in this study

Strain(s) or plasmid	**Relevant characteristics**	**Source or reference***
E. coli strains		
DH5a	F–, ø80dlacZDM15, D(*lacZYA-argF*)U169, *deoR, recA1, endA1, hsdR17* （rK^-,mK^+), *phoA,supE44, l–, thi-1, gyrA96, relA1*	Laboratory collection
BL21	F–,*ompT, hsdS (rBB-mB-), gal,dcm* (DE3)	CWBIO
BL21/pET28a	Kan^r; BL21 containing the pET28a vector	This study
BL21/pET28a-*osaR*	Kan^r; BL21 containing the pET28a-*osaR* vector	This study
BL21/pETMAL-*osaR*	Kan^r; BL21 containing the pETMAL-*osaR* vector	This study
Transetta	*F–ompT hsdSB (rB– mB–) gal dcm lacY1 ahpC* (DE3) gor522::Tn10 *trxB* pRARE	TransGen
Transetta/pET28a-*PA0055*	Kan^r; Transetta containing the pETMAL-*PA0055* vector	This study
P. aeruginosa strains		
PAK(WT)	wild-type strain	David Bradley[a]
△*osaR*	PAK △*osaR(PA0056)*	(1)
△*osaR* / *osaR*	△*osaR* /pDN18- *osaR,* Tc^r; △*osaR* containing the pDN18-*osaR* vector	This study
PAK/pDN18	PAK/ pDN18, Tc^r; △*osaR* containing the pDN18 vector	This study
△*osaR*/pDN18	△*osaR* / pDN18, Tc^r; △*osaR* containing the pDN18 vector	This study
△*dsbM*	PAK △*dsbM* (*PA0058*)	(2)
△*dsbM*/*dsbM*	△*dsbM*/pDN18-*dsbM,*Tc^r;△*dsbM* containing the pDN18-*dsbM* vector	This study
△*dsbM* /pDN18	△*osaR* / pDN18, Tc^r; △*osaR* containing the pDN18 vector	This study
△*osaR*△*dsbM*	PAK △*osaR*△*dsbM*	This study
△*oxyR*	PAK △*oxyR*	This study
△*oxyR*△*osaR*	PAK △*oxyR*△*osaR*	This study
△*oxyR*△*dsbM*	PAK △*oxyR*△*dsbM*	This study
55OE	PAK/pUCP19-*PA0055,* Cb^r; PAK containing the pUCP19-*PA0055* vector; Overexpression of *PA0055* in WT	This study
56OE	PAK/pUCP19-*osaR*, Cb^r; PAK containing the pUCP19-*osaR* vector; Overexpression of *osaR* in WT	This study
58OE	PAK/pUCP19-*dsbM*, Cb^r; PAK containing the	This study

	pUCP19-*dsbM* vector; Overexpression of *dsbM* in WT	
△*dsbM*/58OE	△*dsbM/dsbM*; △*dsbM* /pUCP19- *dsbM* , Cb^r; △*dsbM* containing the pUCP19-*dsbM* vector； Overexpression of *dsbM* in △*dsbM*	This study
△*osaR*/58OE	△*osaR*/*dsbM*; △*osaR*/pUCP19- *dsbM* , Cb^r; △*osaR* containing the pUCP19-*dsbM* vector; Overexpression of *dsbM* in △*osaR*	This study
PAK/pUCP19	Cb^r; PAK containing the pUCP19 vector	This study
△*osaR*/pUCP19	Cb^r; △*osaR* containing the pUCP19 vector	This study
△*dsbM*/pUCP19	Cb^r; △*dsbM* containing the pUCP19 vector	This study
PAK/Tn7:: P_{PA0057}-*gfp*	PAK with P_{PA0057}-*gfp* inserted on chromosome with mini-Tn7T insertion; Gm^r	This study
△*osaR* /Tn7:: P_{PA0057}-*gfp*	△*osaR* with P_{PA0057}-*gfp* inserted on chromosome with mini-Tn7T insertion; Gm^r	This study
Plasmids		
pET28a	Kan^r; vectors carry an N-terminal His-Tag/thrombin/T7-Tag configuration plus an optional C-terminal His-Tag sequence	Novagen
pETMALc-H	Kan^r; maltose-binding protein (MBP) fusion expression vector	(3)
pDN18	Tc^r; Broad-host-range plasmid, IncP	(4)
pDN19lacΩ	Sp^r Sm^r Tc^r; Promoterless *lacZ* fusion vector	(4)
pUCP19	Ap^r; Broad-host-range shuttle vector	(5)
pMH305	Gm^r; pUCP22NotI-based expression vector carrying a promoterless gfpmut3b* gene	(6)
pUC18T-mini-Tn7T-Gm	mini-Tn7 base vector from insertion into chromosome attTn7 site; Gm^r	(7)
pET28a-*osaR*	Kan^r; *osaR* cloned into pET28a at *Nco* I/*Xho* I site	This study
pET28a-*PA0055*	Kan^r; *PA0055* cloned into pET28a at *Nde* I/*Eco* RI site	This study
pETMAL-*osaR*	Kan^r; pETMALc-H vector carrying the malE-*osaR* fusion at *Bam* HI/*Hind* III.	This study
pDN18- *osaR*	Tc^r; *osaR* gene cloned into pDN18 for complement of *osaR* at *Eco* RI/*Hind* III.	This study
pDN18-*dsbM*	Tc^r; *dsbM* gene cloned into pDN18 for complement of *dsbM* at *Eco* RI/*Hind* III.	This study
pUCP19-*PA0055*	Ap^r; *PA0055* gene cloned into pUCP19 for overexpression of PA0055 at *Hind* III/*Eco* RI.	This study
pUCP19- *osaR*	Ap^r; *osaR* gene cloned into pUCP19 for overexpression of *osaR* at *Hind* III/*Eco* RI.	This study
pUCP19- *osaR-his*	Ap^r; *osaR-his* tag fusion cloned into pUCP19	This study

	for overexpression of *osaR* at *Hind* III/*Eco* RI.	
pUCP19-*dsbM*	Ap^r; *dsbM* gene cloned into pUCP19 for overexpression of *dsbM* at *Hind* III/*Eco* RI.	This study
P_{katA}-*gfp*	Gm^r; pMH305 vector carrying the P_{katA}-gfp fusion at *Eco* RI/*Kpn* I.	This study
P_{katB}-*gfp*	Gm^r; pMH305 vector carrying the P_{katB}-gfp fusion at *Eco* RI/*Kpn* I.	This study
P_{oxyR}-*gfp*	Gm^r; pMH305 vector carrying the P_{oxyR}-gfp fusion at *Eco* RI/*Kpn* I.	This study
P_{osaR}-*lacZ*	Sp^r Sm^r Tc^r; P_{osaR}-*lacZ* fusion reporter in pDN19lacΩ at *Eco* RI/*Bam* HI.	This study
P_{PA0057}-*lacZ*	Sp^r Sm^r Tc^r; P_{PA0057}-*lacZ* fusion reporter in pDN19lacΩ at *Eco* RI/ *Bam* HI.	This study
pTn7-P_{PA0057}-*gfp*	Gm^r; pUC18T-mini-Tn7T-Gm vector carrying the P_{PA0057}-gfp fusion at *Eco* RI/*Sac* I.	This study

[a] Faculty of Medicine, Memorial University of Newfoundland, Canada A1B 3V6

Table S2. PCR primers used in this study

Gene (purpose)	**size (bp)**	**Primer name**	**Primer sequence**
osaR(knock sequencing)	1000	P0056YF	5'- ACCAGCAGTTCCGAGGACACG-3'
		P0056YR	5'-ACTCCAGCGCAGCACCATCA -3'
dsbM(knock sequencing)	1000	P0058YF	5'-CCGACCCACTCCTCCAACTC-3'
		P0058YR	5'-TCCAGATAACTGCCGTCACTCC-3'
PA0055(overexpression)	580	PA0055OEF	5'-CCCAAGCTTGATGTCCCTGTCGATCTACCA-3'
		PA0055OER	5'-CCGGAATTCGGGTTTTCCGATCCATTC-3'
osaR (overexpression)	996	PA0056OEF	5'-CCCAAGCTTGATGGACCGCCTGACCG-3'
		PA0056OER	5'-CCGGAATTCCCAGACAGGGGATAAAGGTG-3'
dsbM(overexpression)	858	PA0058OEF	5'-CCCAAGCTTGATGAACGACCTCACCCTG-3'
		PA0058OER	5'-CCGGAATTCCGCCGATGCCGTCTTCT-3'
osaR (complement)	989	PA0056CF	5'-CCGGAATTCCATGGACCGCCTGACCGCC-3'
		PA0056CR	5'-CCCAAGCTTGGGGATAAAGGTGCGTCG-3'
dsbM (complement)	858	PA0058CF	5'-CCGGAATTCCATGAACGACCTCACCCTGC-3'
		PA0058CR	5'-CCCAAGCTTCGCCGATGCCGTCTTCTTC-3'
osaR (expression)	938	PA56EF	5'-
		PA56ER	CATGCCATGGGCATGGACCGCCTGACCGCCACT CGCG-3'
			5'-CCGCTCGAGCGCCAGCGCCAGGGGGCCCGC-3'
malE-osaR(fusion expression)	949	3RH-PA56F	5'-CGCGGATCCATGGACCGCCTGACCGC-3'
		3RH-PA56R	5'-CCCAAGCTTTCCAGCAGTCTACCCGGCTT-3'

*malE-osaR(*fusion expression sequencing)		CX-RHPA56F	5'-GGTCGTCAGACTGTCGATGAAGCC-3'
PA0055(qPCR)	181	PA0055RTF	5'-GAGCTACGCGGATACGGAAAC-3'
		PA0055RTR	5'-GGCGAAGCCGAAGAGGAA-3'
PA0057(qPCR)	176	PA0057RTF	5'-GGGCCGATCCTCAAGGACAAC-3'
		PA0057RTR	5'-ACCAGCACGCCACCAACCAC-3'
dsbM(qPCR)	113	PA0058RTF	5'- GCCTGCGAGGTGACGGGTCTGG -3'
		PA0058RTR	5'- AGGTCATGGGGCATCACGTAGTGGC -3'
rpsL(qPCR)	150	rspLRTF	5'-ACGTGCCTGCGCTGCAAAC-3'
		rspLRTR	5'-CCCGAGGTGTCCAGCGAACC-3'
osaR F1(EMSA)	297	PA56J1F	5'-CGAGACCATCGCCCGGGACA-3'
		PA56J1R	5'-GTTCGGCGCTGGCGGCGAAG-3'
osaR F2IR(EMSA)	238	PA56F2JF	5'-CCTCCAACTCGGCGAGGTAGCG-3'
		PA56F2JR	5'-GCGACATGAGCGGTTCTCCAGG-3'
osaR IR(EMSA)	108	5657JGF	5'-TTCGCTCGATCCATGCAACAAAG-3'
		5657JGR	5'-CATGAGCGGTTCTCCAGGCAGT-3'
osaR F2IRC(EMSA)	90	F2JGMF	5'-CGATGACTTCGACGAAGACG-3'
		F2JGMR	5'- AAAAGCCCCGCAATAATCAC-3'
osaR F3(EMSA)	110	PA56J4F	5'-GCGCCATGGATTGCTTCGTT-3'
		PA56J4R	5'-CGGGGTTGTAGACCTCCAGCC-3'
EMSAc	185	EMSAcF	5'-GCTGTCCCATGAACTACGCC-3'
		EMSAcR	5'-GCCAGGATGTCCCAACTGAA-3'
*flp*JG(EMSA)	459	flpF	5'-GCCGAGGTATCCGAGCAACA -3'
		flpR	5'- GCAATCACCGAGACAGAGCC-3'
P_{PA0057}(promoter-lacZ)	421	P0057F	5'-CCGGAATTCCCGACCCACTCCTCCA-3'
		P0057R	5'-CGCGGATCCTTAGCCCACCAGCAGTTCC-3'
P_{osaR} (promoter-lacZ)	420	P0056F	5'-CCGGAATTCGCCCACCAGCAGTTCC-3'
		P0056R	5'-CGCGGATCCTTAGACCCACTCCTCCAACT-3"
oxyR(qPCR)	192	oxyR-F	5'-TCTTCGAGCGCAGCAAGAGCG-3'
		oxyR-R	5'-GAATCAGGTGCGGGAACAGGTAGG-3'
katA(qPCR)	124	katA-F	5'-GGCTTCTTCGAGCTGAACCGTAAC-3'
		katA-R	5'-GACGGCCCTGGAGCATCTTGTC-3'
katB(qPCR)	103	katB-RTF	5'-CCGGTATTCGTGCGCTTCTCCG-3'
		katB-RTR	5'-TGCCGTCTGCGGTATAGAACTTGGTG-3'
ahpB(qPCR)	188	ahpB-F	5'-GCCTTGCGTGCTTCGTTCCTG-3'
		ahpB-R	5'-GCGCTCGCCTTCATGCCTTTCTG-3'
ahpC(qPCR)	96	ahpC-RTF	5'-GCAAGTGGTCGGTCCTGATCTTCA-3'
		ahpC-RTR	5'-TCTGGAACTCGCCGTAGTTGTTGG-3'
ahpF(qPCR)	115	ahpF-RTF	5'-GGCATCGAGGGCACCTTCGAGTTC-3'
		ahpF-RTR	5'-CGTGACGGATGTTCGGGTTGAGC-3'
*katA*JG1(EMSA)	208	katAJG1F	5'-AAGCTACGTTCGGATAAGCGAG -3'
		katAJG1R	5'- TGCACGTTCTGGTTATCGACC-3'

*katA*JG2(EMSA)	231	katA JG2F	5'-GTCGGCGGTGAAGTCGTAGAAGC -3'
		katA JG2R	5'-ACAACCTGTCGGCTGAACCAAGC-3'
*katB*JG1(EMSA)	235	katB JG1F	5'- ACTTCGCAGGCAAATAAGAAGGT-3'
		katB JG1R	5'- TGGAAGAGCTCCTAATGGCTTGT -3'
*katB*JG2(EMSA)	334	katBJG2F	5'- AATTCACTCAGAAGACCCATGG -3'
		katBJG2R	5'- TTATTTGCCTGCGAAGTTAGG -3'
*oxyR*JG(EMSA)	137	oxyRJGF	5'-GAAGCCGTCTCCTTCCTACAAC-3'
		oxyRJGR	5'-GGCTGCTCATCCGTTAAGATCG-3'
*ahpB*JG(EMSA)	237	ahpBJGF	5'-GGAATAGCCAGAGCAGGCGTGT-3'
		ahpBJGR	5'-TGTTGACCAGTACGCTCATCGC -3'
*ahpF*JG(EMSA)	175	ahpFJGF	5'- ACCTGGTCGGCAAGATCTAAGACCG-3'
		ahpFJGR	5'-GCGTCCAACATGGTTCAGCTCCTC-3'
*ahpC*JG1(EMSA)	203	ahpCJG1F	5'-GCACTGACGAGGGCAGGTTCTT-3'
		ahpCJG1R	5'-AACGGTTGGACTTGAGTGTTGATC-3'
*ahpC*JG2(EMSA)	217	ahpCJG2F	5'-GCTGCTCGGTGCCTGGTTGT-3'
		ahpCJG2R	5'- AACCTGCCCTCGTCAGTGCC -3'
katA(promoter-gfp)	398	katAPF	5'-CCGGAATTCGTCGGCGGTGAAGTCGTAGAA-3'
		katAP2R	5'-CGGGGTACC TTACTGCACGTTCTGGTTATCGACC-3'
katB(promoter-gfp)	564	katBPF	5'- CCGGAATTCAATTCACTCAGAAGACCCATG-3'
		katBP2R	5'-CGGGGTACC GGAAGAGCTCCTAATGGCT-3'
oxyR(promoter-gfp)	155	oxyRPF	5'-CCGGAATTC GAAGCCGTCTCCTTCCTACAAC-3'
		oxyRP2R	5'-CGGGGTACC GGCTGCTCATCCGTTAAGATCG-3'
oxyR(knock out)	424	oxyR-upF	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTC ATGCAACCAGCCCAGCACATG-3'
		oxyR-upR	5'-TCACATGGCTGCTCATCCGTTAAGATC-3'
oxyR(knock out)	474	oxyR-doF	5'-TGACCGAGCTGTCCAGGGTCCC-3'
		oxyR-doR	5'-GGGGACCACTTTGTACAAGAAAGCTGG GTAGCTGCTGGGTGAGTCCTTCG-3'
oxyR(knock sequencing)	1990/1330	oxyRseq-F	5'-TAGACCCCGGTGCTGGAGACG-3'
		oxyRseq-R	5'-CGAGCGGGCCAAGTTGGTAAT-3'

Table S3. MIC of *P. aeruginosa* PAK, Δ*osaR*, Δ*dsbM* and Δ*osaR*Δ*dsbM* against non-aminoglycosides antibiotics investigated.

Bacterial strain	Relevant genotype	MIC of compound (μg ml^{-1})		
		Chloramphenicol	Tetracycline	Ciprofloxacin
PAK	WT	50	16	0.5
Δ*osaR*	Δ*osaR*	50	16	0.5
Δ*dsbM*	Δ*dsbM*	50	16	0.5
Δ*osaR*Δ*dsbM*	Δ*osaR*Δ*dsbM*	50	16	0.5

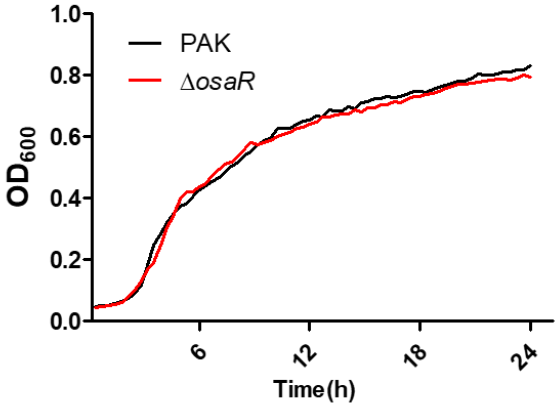

Fig. S1. The knocking out of *osaR* have no effect on growth rate. Overnight culture were diluted into fresh LB medium with 1/100, 100μl of culture was transferred into each of the well of a 96-wells microplate, OD_{600} was measured every 30 minutes for 24 hours at 37℃ 200 rpm in microplate reader.

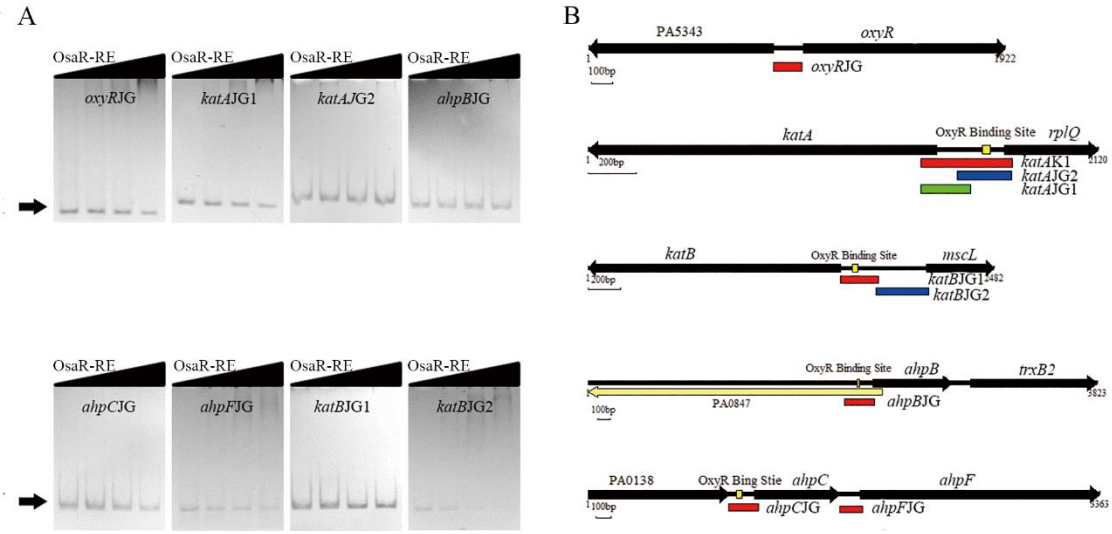

Fig. S2. The regulation of *oxyR* and the genes belonging to the OxyR regulon by OsaR is indirect. (A)EMSA assays of OsaR-RE binding with promoter region of *oxyR, katA, katB, ahpB* and *ahpCF*; DNA fragments information are shown in (B). OsaR was used in the following concentration (nM): 0, 50, 100, 200. Black arrows indicate free DNA.

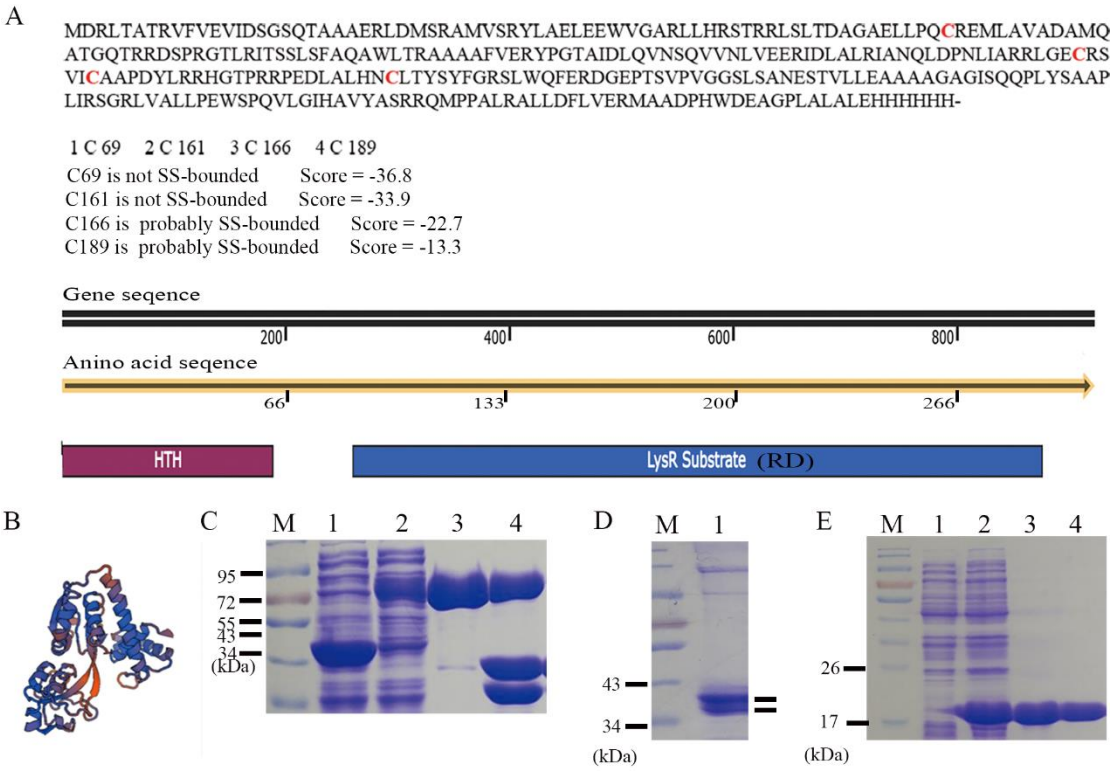

Fig. S3. Expression and purification of OsaR protein (C-D) and PA0055 protein(E). (A) the amino sequence of OsaR, which have four cysteines in red color. C166 and C189 are located at regulate domain(RD) and form disulfide bond probably. (B) OsaR tertiary structure prediction. OsaR was expressed in BL21(DE3) host strain via pET28a insolubility (C) MBP- OsaR fusion expression (Lane 2), purification (Lane 3), cleavage(Lane 4) and Lane 1 is MBP expression control as followed by SDS-PAGE. (D) nonreducing SDS-PAGE of OsaR protein（purified with inclusion body; M:Protein marker. (E)PA0055 protein expression in the BL21(DE3) strain with pET28a vector. Lane 1, negative control; Lane 2 cell lysate supernatant; Lane 3, acquired pure PA0055 protein in 200 mM imidazole solution; Lane 4, pure PA0055 after ultra-filtration desalination). The same molecular weight marker was used for all SDS–PAGE analyses.

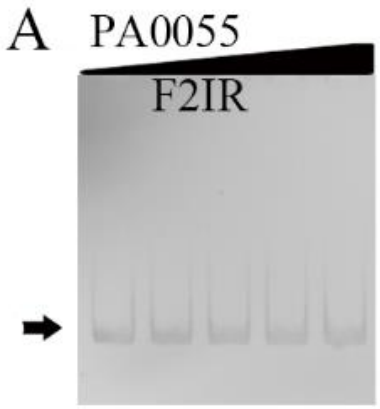

Fig. S4. The EMSA of PA0055 protein with F2IR. PA0055 used in the following concentration (nM) 0, 50, 100, 200, 400. All the data were obtained from at least three independent experiments.

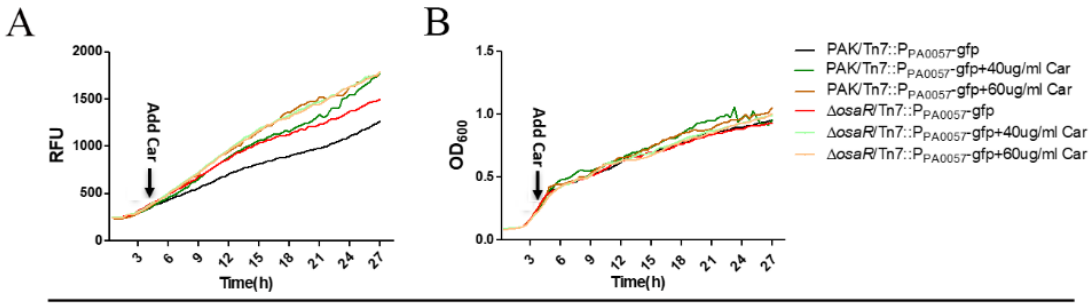

Fig. S5. Promoter P_{PA0057} can be induced by carbenicillin. Bacteria were treated with given concentration of carbenicillin at the OD_{600} of 0.4 in LB at 37°C. (B) OD_{600} and (A) GFP fluorescence readings (relative fluorescence units/RFU) were measured every 30 minutes for 24 hours. All the data were obtained from at least three independent experiments with at least three replicates.

REFERENCE

1. Han Xiao LY, Ma Yibing, Xu Haijin, Qiao Mingqiang 2019. Interaction between Disulfide Oxidoreductase (DsbM) and LysR Type Transcriptional Regulator (PA0056) in Pseudomonas aeruginosa Acta Scientiarum Naturalium Universitatis Nankaiensis 52:6.
2. Wang X, Li M, Liu L, Mou R, Zhang X, Bai Y, Xu H, Qiao M. 2012. DsbM, a novel disulfide oxidoreductase affects aminoglycoside resistance in Pseudomonas aeruginosa by OxyR-regulated response. J Microbiol 50:932-8.
3. Pryor KD, Leiting B. 1997. High-Level Expression of Soluble Protein inEscherichia coliUsing a His6-Tag and Maltose-Binding-Protein Double-Affinity Fusion System. Protein Expression and Purification 10:309-319.

4. Totten PA, Lory S. 1990. Characterization of the type a flagellin gene from Pseudomonas aeruginosa PAK. Journal of Bacteriology 172:7188-7199.
5. Schweizer HP. 1991. Escherichia-Pseudomonas shuttle vectors derived from pUC18/19. Gene 97:109-112.
6. Rybtke MT, Borlee BR, Murakami K, Irie Y, Hentzer M, Nielsen TE, Givskov M, Parsek MR, Tolker-Nielsen T. 2012. Fluorescence-Based Reporter for Gauging Cyclic Di-GMP Levels in Pseudomonas aeruginosa. Applied and Environmental Microbiology 78:5060-5069.
7. Choi KH, Schweizer HP. 2006. Mini-Tn7 insertion in bacteria with single attTn7 sites: example Pseudomonas aeruginosa. Nature Protocols 1:153-161.