Functional characterization of TetR-like transcriptional regulator PA3973 from *Pseudomonas aeruginosa*

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SUPPLEMENTARY MATERIALS

Table S1. Bacterial strains, plasmids used and constructed in this study.

strain	description	reference
Escherichia coli		
DH5α	F⁻ Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*) *U169 recA1 endA1 hsdR17* (r_{K-}, m_{K+}) *phoA supE44 λ thi-1 gyrA96 relA1*	[1]
S17-1	*pro* Δ*hsdR hsdM*⁺ *recA* Tp^R Sm^R ΩRP4-Tc::Mu Kn::Tn7	[2]
BL21	F⁻ *ompT hsdS*$_B$ ($r_B^- m_B^-$) *gal dcm* (λ DE3)	Novagen
Pseudomonas aeruginosa		
PAO1161	PAO1161 Rif^R *leu*⁻, r⁻, m⁻	[3]
PAO1161	PAO1161 Rif^R r⁻, m⁻	[4]
PAO1161 Δ*PA3973*	PAO1161 Rif^R with deleted gene *PA3973*, allele exchange with the use of pKKB60.6	This study
PAO1161 Δ*PA3972-71*	PAO1161 Rif^R with deleted genes *PA3972* and *PA3971*, allele exchange with the use of pSOB3	This study
plasmid	**description**	**reference**
pBBR1-MCS-1	Cm^R, IncA/C broad-host-range cloning vector, *lacZα*–MCS, *mob*, T7p, T3p	[5]
pAMB9.37	pBBR1-MCS-1 derivative with *lacI*^{Q} *tac*p, expression vector	[6]
pABB28.1	pBBR1-MCS-1 derivative with *lacI*^{Q} *tac*p-*flag*, expression vector	[7]
pAKE600	Ap^R, *ori*$_{MB1}$, *oriT*$_{RK2}$, *sacB*, suicide vector	[8]
pKAB240	pUC19 derivative with *his*$_6$-*mcs* (MunI, HindIII, NotI, XhoI, BamHI)-*flag*	[9]
pMEB1	pAMB9.37 derivative with modified *mcs*	[9]
pBGS18	Km^R, *ori*$_{MB1}$, cloning vector	[10]
pET28a(+)	Km^R, *ori*$_{MB1}$, T7p, *lacO*, His_6-tag, T7 tag, expression vector	Novagen

pPTO1	Km^R, $oriV_{pSC101}$, promoter-less *xylE* cassette	[11]
pET28mod	Km^R, ori_{MB1}, T7p, *lacO*, His_6-tag, modified to remove T7 tag	[12]
pKKB3.11	pAMB9.37 derivative, $lacI^q$-*tac*p-*PA3973*, *PA3973* amplified with primers #1/#2 and cloned using EcoRI/SacI	This study
pKKB60.3	pAKE600 derivative with the fragment upstream of *PA3973* amplified with primers #3/#4 and cloned using BamHI/HindIII	This study
pKKB18.3	pBGS18 derivative with the fragment downstream of *PA3973* amplified with primers #5/#6 and cloned using HindIII/EcoRI	This study
pKKB60.6	pAKE600 derivative with fragments upstream and downstream of *PA3973* obtained by re-cloning of *PA3973* downstream region from pKKB18.3 to pKKB60.3 using HindIII/EcoRI	This study
pSOB3.1	pAKE600 derivative with the fragment upstream of *PA3972* amplified with primers #7/#8 and cloned using EcoRI/HindIII	This study
pSOB3.4	pBGS18 derivative with the fragment downstream of *PA3971* amplified with primers #9/#10 and cloned using HindIII/ BamHI	This study
pSOB3	pAKE600 derivative with fragments upstream and downstream of *PA3972-71* obtained by re-cloning of *PA3971* downstream region from pSOB3.4 to pSOB3.1 using HindIII/BamHI	This study
pKKB28.3	pET28mod derivative encoding His_6-PA3973, *PA3973* was amplified with primers #1/#2 and cloned using EcoRI/SacI	This study
pMEB265	pET28mod derivative encoding PA3973-His_6, *PA3973* was amplified with primers #1/#11 and cloned using EcoRI/XhoI	This study
pMEB251	pKAB240 derivative with *PA3973* gene without STOP codon amplified with primers #1/#13 and cloned using	This study

	EcoRI/HindIII	
pMEB255	pMEB1 derivative encoding *lacI*^{q}-*tac*p-*PA3973-flag*, *PA3973-flag* re-cloned from pMEB251 using EcoRI/SalI	This study
pMEB267	pPTOI derivative with *PA2468*p-*xylE*, *PA2468*p amplified with primers #19/#20 and cloned using SphI/BamHI	This study
pMEB269	pPTOI derivative with *PA4156*p-*xylE*, *PA4156*p amplified with primers #23/#24 and cloned using SphI/BamHI	This study

Table S2 List of primers used in this study.

nr	name	sequence 5´-3´
#1	3973eF	gcgaattcATGGTCTATCGTGTCACCG
#2	3973eR	gcgagctcTGCAGGTTCATGAGGGTTC
#3	3973mLF	gcggatcCCGGTCAAGTTCGAAGAGTT
#4	3973mLR	gcaagcttAGACCATGACTGAATCCG
#5	3973mPF	gcaagcttAATAGAGGAACCCTCATGAACCTGCAC
#6	3973mPR	gcgaattcCTCCGGCTTGTGCTGGTTGG
#7	3972pF	gcgaattcAGCCAGCGTGAGGTCGATGC
#8	3972upHR	cgcgaagcttCAGGTTCATGAGGGTTCCTC
#9	3972dwHF	cgcgaagcttTGAGGTAACGGGAGAAAAGC
#10	3972dwBR	cgggtaccGGTGACGGTGACGCTGTATTC
#11	3973eR2	gagctcgtgAGGGTTCCTCGCAGA
#12	3973HR	gcaaagcttTGAGGGTTCCTCGCAGACAG
#13	p3973F	gcgaattcGACGGCGTACTGCTCGAC
#14	p3973R*	gcggatccTCACGCTTCAGGCTTTGC
#15	pPA0061F	gcgaattcgCATGCCGGCCCCGTGGACAGCCCGC
#16	pPA0061R	gaggatccGGGGCGGGCTCCGGAGGGT
#17	pPA0195F	gcgaattcgcatgcGATGGGCGGGAATTGTTGG
#18	pPA0195R	gcggatccTCACGAATCTCCTGCGTGA
#19	pPA2468F	gcgaattcgcatgcGGCGCTGGAGATTCCCGGC
#20	pPA2468R	gcggatccCATGGGAAAGTCGGGGCGA
#21	pPA2722F	gagaattcgcatgcCAATGGCGGCGAGGAAAGC
#22	pPA2722R	gcggatccTTCGTGACTCCTTTGCAAG
#23	pPA4156F	gcgaattcgcatgcCACCACGGTTGATCCATAG
#24	pPA4156R	gcggatccAGGATCTTCTCCAAATGGG
#25	pPA4710F	gcgaattcgcatgcGCTCGGCAGGGGAATGGGA
#26	pPA4710R	gaggatccGTGGGACTCCTTGGGTCGG
#27	TproPIIa	CATGTGGTACCATAATAGTTAACGAGAACCCCGG CAGCTGCCGGGGTTATTTTTGGTGGTTCCATGGC
#28	TproPIIb	CATGGCCATGGAACCACCAAAAATAACCCCGGCA GCTGCCGGGGTTCTCGTTAACTATTATGGTACCA
Primers used in RT-qPCR		
#29	qPA3973F	GGATCCTGAAGTCGACGAGC
#30	qPA3973R	GAAAGCTGGAATGCGCCAC
#31	qPA3972F	GGGCAAGTACTGGATCTGCA
#32	qPA3972R	CGGAACCTTCCCAGATCGAG
#33	qPA3971F	CAGAACGGCTTCATCCATGC

#34	qPA3971R	TTGAACTCCAGGGTCAGCAC
#35	qPA3970F	AGCGGCAGAACTTCCACTACCC
#36	qPA3970R	GGTGACGGTGACGCTGTATTCG
#37	PA0671qF	GTCGGCGAACTGCAACTAC
#38	PA0671qR	CTGCGGATAGGGTACGTAGG
#39	qPA3614F	AACTCATGCTGCTGGACTCC
#40	qPA3614R	TGGGTATACAGCGGCTTGATG
#41	PA5208qF	CAGGACAAAGTCGCCAATCG
#42	PA5208qR	GAGCATCTGGCCTTGTAGCG
#43	PA5460qF	CTGCCCATCCACATCTCGCC
#44	PA5460qR	AGGTCGTGTTCGGACATTTG
#45	PA5497qF	GGGACAAGAAGTACCGGCTC
#46	PA5497qR	GAGGCCTTGTCCTCGACATC
#47	PA2174qF	AACTGAACCCCGACTTCACG
#48	PA2174qR	GAAGCTGCTGCTCTTCAGGA
#49	D3C65_10195qF	CATTATGGACTTTCGCGCCG
#50	D3C65_10195qR	TTACAGGCGAATGCGACCAC
#51	PROCF	CAGGCCGGGCAGTTGCTGTC
#52	PROCR	GGTCAGGCGCGAGGCTGTCT

Table S3. RNA-seq data for PA3973+ and EV transcriptomes [fold change (FC) ≤ -2 or ≥ 2, FDR adjusted P ≤ 0.01]. Genes identified only in strain PAO1161 but not in PAO1 are described as "not annotated (NA)".

Table S4. Results of ChIP-seq analysis – intergenic regions. 139 PA3973-FLAG ChIP-seq peaks with a fold enrichment (FE) cut-off value of ≥ 2 [FDR<0.01] identified in intergenic regions. RNA-seq data for Δ*PA3973* vs WT strain presented as a fold change (FC) are included. Genes identified only in PAO1161 strain but not in PAO1 are described as "not annotated (NA)".

Table S5. Results of ChIP-seq analysis – coding regions. 179 PA3973-FLAG ChIP-seq peaks identified in coding regions with a fold enrichment (FE) cut-off value of ≥ 2 [FDR<0.01], obtained by the comparison of PA3973-FLAG ChIP samples with negative control samples. RNA-seq data for Δ*PA3973* vs WT strain presented as a fold change (FC) are included. Genes identified only in PAO1161 strain but not in PAO1 are described as "not annotated (NA)".

Table S6. RNA-seq data for transcriptomes of PA3973-deficient cells vs. WT strain [fold change (FC) ≤ -2 or ≥ 2, FDR adjusted P ≤ 0.01]. RNA-seq data for PA3973+ vs EV+ are presented for comparison. Gene identified only in strain PAO1161, but not in PAO1 is described as "not annotated (NA)".

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