DECIPHERING THE CHE2 CHEMOSENSORY PATHWAY AND THE ROLES OF INDIVIDUAL CHE2 PROTEINS FROM *PSEUDOMONAS AERUGINOSA*

SUPPLEMENTARY INFORMATION

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Running title: Che2 interactions in *P. aeruginosa*

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

Bacterial strains	Genotype or description	Reference or source
P. aeruginosa		
PAO1 (mPAO1)	WT *P. aeruginosa*	(Jacobs *et al.*, 2003, Held *et al.*, 2012)
PW1295 (PAO1 *cheB2*)	mPAO1 with transposon ISphoA/hah inserted at nt 218 of PA0173	(Jacobs *et al.*, 2003, Held *et al.*, 2012)
PW1297 (PAO1 *cheD*)	mPAO1 with transposon ISlacZ/hah inserted at nt 28 of PA0174	(Jacobs *et al.*, 2003, Held *et al.*, 2012)
PW1299 (PAO1 *cheR2*)	mPAO1 with transposon ISphoA/hah inserted at nt 109 of PA0175	(Jacobs *et al.*, 2003, Held *et al.*, 2012)
E. coli		
BL21(DE3)	*F⁻ ompT gal dcm lon hsdS*$_B$(*r*$_B$- *m*$_B$-) (λDE3)	
BTH101	*F⁻ cya-99 araD139 galE15 galK16 rpsL1 (Str*^{R}*) hsdR2 mcrA1 mcrB1*	Euromedex
BT3388	*tar tsr trg tap aer*	(Yu *et al.*, 2002)
UU2610	*tar tsr trg tap aer cheR cheB*	(Zhou *et al.*, 2011)
Plasmids	**Description or construction**	**Reference or source**
P. aeruginosa		
pJN105	Broad host range plasmid with Gm^R and *araC-P*$_{BAD}$	(Newman & Fuqua, 1999)
pJN105-Aer2	*his-aer2* (PA0176) amplified from pLH1 with its RBS and cloned into the EcoRI site of pJN105	This study
E. coli		
pProEXHTa	Plasmid containing Amp^R, *lacI*^{q}, and *P*$_{trc}$	Invitrogen
pLH1 (pProEXHTa-Aer2)	pProEXHTa expressing full-length His-Aer2 (Aer2$_{QEEE}$, WT) from *P*$_{trc}$	(Watts *et al.*, 2011)
pProEXHTa-Aer2$_{EEEE}$	Q414E was introduced into pLH1 by site-directed mutagenesis to express His-Aer2$_{EEEE}$	This study
pProEXHTa-Aer2$_{QQQQ}$	E421Q, E428Q, and E610Q were sequentially introduced into pLH1 by site-directed mutagenesis to express His-Aer2$_{QQQQ}$	This study
pProEXHTa-CheA2	CheA2 (PA0178) was amplified from PAO1 DNA and cloned into the NcoI and HindIII sites of pProEXHTa to express His-CheA2	This study
pProEXHTa-CheY2	CheY2 (PA0179) was amplified from PAO1 DNA and cloned into the NcoI and HindIII sites of pProEXHTa to express His-CheY2	This study
pProEXHTa-CheY2-D10K	D10K was introduced into pProEXHTa-CheY2 by site-directed mutagenesis to express His-CheY2-D10K	This study
pProEXHTa-CheY	CheY (PA1456) was amplified from PAO1 DNA and cloned into the NcoI and HindIII sites of pProEXHTa to express His-CheY	This study
pProEXHTa-*Ec*CheA	CheA was amplified from *E. coli* BT3388 DNA and cloned into the NcoI and HindIII sites of pProEXHTa to express His-*Ec*CheA	This study
pProEXHTa-CheD	CheD (PA0174) was amplified from PAO1 DNA and cloned into the NcoI and HindIII sites of pProEXHTa to express His-CheD	This study
pProEXHTa-Aer2$_{380-679}$	pProEXHTa expressing His-Aer2$_{380-679}$	(Watts *et al.*, 2011)
pProEXHTa-Aer$_{260-521}$	Aer$_{260-521}$ (PA1561) was amplified from PAO1 DNA and cloned into the BamHI and SalI sites of pProEXHTa to express His-Aer$_{260-521}$	This study

pProEXHTa-CtpM$_{300-561}$	CtpM$_{300-561}$ (PA2652) was amplified from PAO1 DNA and cloned into the EcoRI and NotI sites of pProEXHTa to express His-CtpM$_{300-561}$	This study
pVSCheY-6H	Amp^R and *lacI^q* plasmid expressing *E. coli* CheY-His	(Miller *et al.*, 2006)
pKG116	Plasmid with Cam^R and P_{nahG}	(Buron-Barral *et al.*, 2006)
pKG116-CheB2	CheB2 (PA0173) was amplified from PAO1 DNA and cloned into the NdeI and BamHI sites of pKG116 to express His-CheB2	This study
pKG116-CheD	CheD (PA0174) was amplified from PAO1 DNA and cloned into the NdeI and BamHI sites of pKG116 to express His-CheD	This study
pKG116-CheD-V41T	V41T was introduced into pKG116-CheD by site-directed mutagenesis to express His-CheD-V41T	This study
pKG116-CheR2	CheR2 (PA0175) was amplified from PAO1 DNA and cloned into the NdeI and BamHI sites of pKG116 to express His-CheR2	This study
pKG116-CheDB2	CheDB2 was amplified from PAO1 DNA and cloned into the NdeI and BamHI sites of pKG116 to express His-CheD and CheB2	This study
pKG116-CheR2D	CheR2D was amplified from PAO1 DNA and cloned into the NdeI and BamHI sites of pKG116 to express His-CheR2 and CheD	This study
pKG116-CheR2DB2	CheR2DB2 was amplified from PAO1 DNA and cloned into the NdeI and BamHI sites of pKG116 to express His-CheR2, CheD and CheB2	This study
pKG116-CheR1	CheR1 (PA3348) was amplified from PAO1 DNA and cloned into the NdeI and EcoRI sites of pKG116 to express His-CheR1	This study
pKG116-CheR1D	CheR1 and CheD were each amplified from PAO1 DNA and cloned into the NdeI and BamHI sites of pKG116 with an EcoRI/linker (11 nts) in between in order to express His-CheR1 and His-CheD	This study
pKT25	Km^R plasmid with P_{lac} encoding the T25 fragment of *B. pertussis cyaA* and a downstream MCS	Euromedex
pKT25-CheA2	CheA2 (PA0178) was amplified from PAO1 DNA and cloned into the XbaI and EcoRI sites of pKT25 to express T25-CheA2	This study
pKT25-CheY2	CheY2 (PA0179) was amplified from PAO1 DNA and cloned into the XbaI and EcoRI sites of pKT25 to express T25-CheY2	This study
pKT25-CheY2-D10K	D10K was introduced into pKT25-CheY2 by site-directed mutagenesis to express T25-CheY2-D10K	This study
pKT25-CheY2/CheA2	*his-cheA2* was amplified from pProEXHTa-CheA2 with its P_{trc} promoter and cloned into the HindIII site of pKT25-CheY2 to express T25-CheY2 and His-CheA2	This study
pKT25-CheY2-D10K/CheA2	D10K was introduced into pKT25-CheY2/CheA2 by site-directed mutagenesis to express T25-CheY2-D10K and His-CheA2	This study
pKT25-CheR2	CheR2 (PA0175) was amplified from pKG116-CheR2 and cloned into the XbaI and EcoRI sites of pKT25 to express T25-CheR2	This study
pKNT25	Km^R plasmid with P_{lac} containing a MCS upstream of the T25 fragment of *B. pertussis cyaA*	Euromedex
pKNT25-CheY2	CheY2 (PA0179) was amplified from PAO1 DNA and cloned into the HindIII and XbaI sites of pKNT25 to express CheY2-T25	This study

pKNT25-CheY2-D10K	D10K was introduced into pKNT25-CheY2 by site-directed mutagenesis to express CheY2-D10K-T25	This study
pKNT25-CheY2/CheA2	*his-cheA2* was amplified from pProEXHTa-CheA2 with its P_{trc} promoter and cloned into the HindIII site of pKNT25-CheY2 to express CheY2-T25 and His-CheA2	This study
pKNT25-CheY	CheY (PA1456) was amplified from PAO1 DNA and cloned into the HindIII and XbaI sites of pKNT25 to express CheY-T25	This study
pUT18	Amp^R plasmid with P_{lac} containing a MCS upstream of the T18 fragment of *B. pertussis cyaA*	Euromedex
pUT18-CheA2	CheA2 (PA0178) was amplified from PAO1 DNA and cloned into the XbaI and EcoRI sites of pUT18 to express CheA2-T18	This study
pUT18-CheD	Full-length CheD (PA0174, nts encoding res. 1-200) was amplified from PAO1 DNA and cloned into the XbaI and EcoRI sites of pUT18 to express CheD-T18	This study
pUT18-CheD$_{1-182}$	CheD [nts encoding res. 1-182] was amplified by inverse PCR of pUT18-CheD and ligated at EcoRI to express CheD$_{1-182}$-T18	This study
pUT18-CheD$_{1-167}$	CheD [nts encoding res. 1-167] was amplified by inverse PCR of pUT18-CheD and ligated at EcoRI to express CheD$_{1-167}$-T18	This study
pUT18C	Amp^R plasmid with P_{lac} encoding the T18 fragment of *B. pertussis cyaA* and a downstream MCS	Euromedex
pUT18C-CheA2	CheA2 (PA0178) was amplified from PAO1 DNA and cloned into the XbaI and EcoRI sites of pUT18C to express T18-CheA2	This study
pUT18C-*Ec*CheA	CheA was amplified from *E. coli* BT3388 DNA and cloned into the PstI and BamHI sites of pUT18C to express T18-*Ec*CheA	This study
pUT18C-FliM	FliM (PA1443) was amplified from PAO1 DNA and cloned into the XbaI and EcoRI sites of pUT18C to express T18-FliM	This study
pUT18C-CheD	CheD (PA0174) was amplified from PAO1 DNA and cloned into the XbaI and EcoRI sites of pUT18C to express T18-CheD	This study
PAO1 libraries	**Description**	**Source**
pUT18-PAO1lib	pUT18 containing a PAO1 genomic library as an N-terminal fusion to T18 (see methods for details)	This study
pUT18C-PAO1lib	pUT18C containing a PAO1 genomic library as C-terminal fusion to T18 (see methods for details)	This study

Table S2. Summary of *P. aeruginosa* PAO1 gDNA library screens with CheY2

Screen	Co-transformants	Red clones	Red after restreaking	CheY2 partners
pKT25-CheY2/CheA2 + pUT18-PAO1lib pUT18C-PAO1lib **total**	391, 500 342, 100 **733, 600**	79 258 **337**	22 11 **33**	- 2 **2**
pKNT25-CheY2/CheA2 + pUT18-PAO1lib pUT18C-PAO1lib **total**	216, 808 39, 680 **256, 488**	108 153 **261**	30 1 **31**	- - -
pKT25-CheY2-D10K + pUT18-PAO1lib pUT18C-PAO1lib **total**	31, 560 355, 840 **387, 400**	2 304 **306**	- 17 **17**	- - -

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