

## Chemotaxis proteins and transducers for aerotaxis in *Pseudomonas aeruginosa*

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### Abstract

It was previously shown that the chemotaxis gene cluster 1 (*cheYZABW*) was required for chemotaxis. In this study, the involvement of the same cluster in aerotaxis is described and two transducer genes for aerotaxis are identified. Aerotaxis assays of a number of deletion–insertion mutants of *Pseudomonas aeruginosa* PAO1 revealed that the chemotaxis gene cluster 1 and *cheR* are required for aerotaxis. Mutant strains which contained deletions in the methyl-accepting chemotaxis protein-like genes *tlpC* and *tlpG* showed decreased aerotaxis. A double mutant deficient in *tlpC* and *tlpG* was negative for aerotaxis. TlpC has 45% amino acid identity with the *Escherichia coli* aerotactic transducer Aer. The TlpG protein has a predicted C-terminal segment with 89% identity to the highly conserved domain of the *E. coli* serine chemoreceptor Tsr. A hydropathy plot of TlpG indicated that hydrophobic membrane-spanning regions are missing in TlpG. A PAS motif was found in the N-terminal domains of TlpC and TlpG. On this basis, the *tlpC* and *tlpG* genes were renamed *aer* and *aer-2*, respectively. No significant homology other than the PAS motif was detected in the N-terminal domains between Aer and Aer-2. © 2004 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

**Keywords:** Aerotaxis; *Pseudomonas aeruginosa*; Chemotaxis; Behavioral response; Transducer

### 1. Introduction

*Pseudomonas aeruginosa* is an obligately aerobic bacterium and is capable of swimming by rotating a single polar flagellum. This organism inhabits a wide range of environments, from soil and water to the human host. It is an opportunistic pathogen that is among the most frequently isolated bacteria in nosocomial infections [1]. *P. aeruginosa*, like most other motile bacteria, has chemotactic responses to a wide range of chemical stimuli. *P. aeruginosa* is attracted to 20 commonly occurring L-amino acids [2,3], sugars [4], organic acids [5], and inorganic phosphate [6]. *P. aeruginosa* is repelled by thiocyanic and isothiocyanic esters [7] and volatile chlorinated aliphatic compounds such as trichloroethylene, tetrachloroethylene, trichloroethane, and chloroform [8]. Chemotaxis

is a clear indicator of bacterial responses to changing environments. It can be viewed as an important prelude to metabolism, symbiosis, and other ecological interactions in microbial communities [9].

Aerotaxis is the movement of a cell towards or away from oxygen. Bacteria use aerotaxis to swim toward an optimal oxygen concentration for their metabolism. Aerotaxis has been extensively investigated in the facultative anaerobe *Escherichia coli*. In *E. coli*, an active respiratory chain and chemotaxis (Che) proteins such as CheA, CheY, and CheW are required and Aer and Tsr function as independent sensor/transducers for aerotaxis [10–12]. Aer is a methyl-accepting chemotaxis protein (MCP)-like transducer which contains a PAS (an acronym of the *Drosophila* period clock protein [PER], vertebrate aryl hydrocarbon receptor nuclear translocator [ARNT], and *Drosophila* single-minded protein [SIM]) motif in the N-terminal domain [13]. The PAS motif comprises a binding pocket for a prosthetic group [14] and Bibikov et al. [12] showed that Aer contained high levels of non-covalently associated flavin adenine dinucleotide (FAD). It is postulated that Aer

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Table 1  
Bacterial strains and plasmids used in this study

Strain/plasmid	Relevant characteristics <sup>a</sup>	Source or reference
<b>Strains</b>		
<i>E. coli</i>		
MV1184	<i>ara</i> Δ( <i>lac-proAB</i> ) <i>rpsL</i> <i>thi</i> (φ80 <i>lacZ</i> ΔM15) Δ( <i>srl-recA</i> )306::Tn10(Tc <sup>r</sup> ) F'[ <i>traD</i> 36 <i>proAB</i> <sup>+</sup> <i>lac</i> <sup>R</sup> <i>lacZ</i> ΔM15]	[31]
HB101	<i>hsdS</i> 20 <i>recA</i> 13 <i>proA</i> 2 <i>leu</i> 6 <i>thi</i> -1 <i>rpsL</i> 20 <i>ara</i> -14	[32]
<i>P. aeruginosa</i>		
PAO1	Prototroph, FP <sup>−</sup> (sex factor minus)	[33]
ΔCHE1	PAO1 derivative, ΔChe cluster 1 ( <i>cheY</i> <i>cheZ</i> <i>cheA</i> <i>cheB</i> PA1460–1463 <sup>b</sup> <i>cheW</i> )	This study
ΔCHE3	PAO1 derivative, ΔChe cluster 3 ( <i>pilJ</i> <i>pilK</i> <i>pilL</i> <i>chpA</i> <i>chpB</i> )	This study
ΔCHE4	PAO1 derivative, ΔChe cluster 4 ( <i>cheY</i> <sub>2</sub> <i>cheA</i> <sub>2</sub> <i>cheW</i> <sub>2</sub> <i>tlpG</i> <i>cheR</i> <sub>2</sub> PA0174 <sup>b</sup> <i>cheB</i> <sub>2</sub> )	This study
ΔCHE5	PAO1 derivative, ΔChe cluster 5 ( <i>cheW</i> <sub>3</sub> <i>cheR</i> <sub>3</sub> <i>cheW</i> <sub>4</sub> <i>cheA</i> <sub>3</sub> <i>cheB</i> <sub>3</sub> )	This study
ΔpilHIJK	PAO1 derivative, ΔpilHIJK cluster ( <i>pilH</i> <i>pilI</i> <i>pilJ</i> <i>pilK</i> )	[24]
PC4	NTG derived mutant of PAO1, <i>cheR</i>	[19]
TLPC1	PAO1 derivative, <i>tlpC</i> :: <i>tet</i>	This study
TLPG1	PAO1 derivative, <i>tlpG</i> :: <i>kan</i>	This study
TLPCG1	PAO1 derivative, <i>tlpC</i> :: <i>tet</i> <i>tlpG</i> :: <i>kan</i>	This study
<b>Plasmids</b>		
pBluescript II KS <sup>+</sup>	General cloning vector; Ap <sup>r</sup>	Stratagene
pUC118	General cloning vector; Ap <sup>r</sup>	[31]
pHSG396	General cloning vector; Cm <sup>r</sup>	Takara Shuzo
pUC4K	pUC4 containing a 1.3-kb <i>kan</i> cartridge; Ap <sup>r</sup> Km <sup>r</sup>	Pharmacia
pUC118Tc	pUC118 containing a 1.3-kb <i>tet</i> cartridge from pBR322; Ap <sup>r</sup> Tc <sup>r</sup>	[16]
pCP19	Broad-host-range cosmid; Tc <sup>r</sup> IncP	[34]
pMRP9-1	Broad-host-range GFP expression vector; Cb <sup>r</sup>	[23]
pPT07.1	pCP19 derivative containing Che cluster 1 from <i>P. aeruginosa</i> PAO1; Tc <sup>r</sup>	[19]
TLPC01.2	pMRP9-1 derivative containing a 3.3-kb <i>tlpC</i> PCR product; Cb <sup>r</sup>	This study
pTLPG01.2	pMRP9-1 derivative with a 2.1-kb <i>SalI</i> fragment containing <i>tlpG</i> from <i>P. aeruginosa</i> PAO1; Cb <sup>r</sup>	This study

<sup>a</sup>Ap, ampicillin; Tc, tetracycline; Km, kanamycin; Cm, chloramphenicol; Cb, carbenicillin; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; GFP, green fluorescent protein.

<sup>b</sup>Gene ID numbers used in the *P. aeruginosa* genome sequencing project (<http://www.pseudomonas.com/>).

#### 2.4.1. Che cluster 1

A 2.0-kb *Clal*-*HpaI* fragment containing *fleN* and *fliA* from pPT07.1 [19], a 1.3-kb *HincII*-flanked *kan* gene cassette (conferring kanamycin resistance [Km<sup>r</sup>]) from pUC4K, and a 2.0-kb *HincII*-*HindIII* fragment including open reading frames (ORFs) PA1465, PA1466, and PA1467 from pPT07.1 were ligated with the backbone of *Clal*/*HindIII*-digested pHSG396 to obtain pPT07.20.

#### 2.4.2. Che cluster 2

The isolation of the *cheR* mutant PC4 has been described previously [19].

#### 2.4.3. Che cluster 3

The deletion of the *pilHIJK* genes has been described previously [24]. A 0.7-kb DNA fragment containing an N- and C-terminally truncated copy of *pilJ* was amplified with the PCR primers *pilJf* and *pilJr* and the PCR product was cloned into pUC118 to make pPT07.21. A 0.9-kb DNA fragment containing the 3' end of *chpB* amplified with the primers *chpBf* and *chpBr* was cloned into pUC118 to obtain pPT07.22. A 0.7-kb *XbaI*-*EcoRI* fragment from pPT07.21, a 1.3-kb *EcoRI*-flanked *kan* gene cassette from pUC4K, and a 0.9-kb *EcoRI*-*SphI* fragment from pPT07.22 were ligated with the backbone of the *XbaI*/*SphI*-digested pUC118 to obtain pPT07.23.

#### 2.4.4. Che cluster 4

A 1.7-kb DNA fragment, which contained the entire *tlpF* gene and the 5' end of *cheY*<sub>2</sub>, was amplified with the PCR primers *tlpFf* and *cheY2r*. The PCR product was cloned into pBluescript II KS<sup>+</sup> to make pPT07.24. A 1.0-kb *SalI*-*BamHI* chromosomal DNA fragment containing the 3' end of *cheB*<sub>2</sub> was cloned into pUC118 to obtain pPT07.25. A 1.7-kb *XbaI*-*XhoI* fragment from

Table 2  
Oligonucleotides used for PCR

Primer	Sequence (5' to 3')
<b>Primers used for construction of ΔCHE3</b>	
<i>pilJf</i>	TTTGCCCTACCTCAACACCCAGTCG
<i>pilJr</i>	GCGAAACGCTGAAGATGTTGTTCCG
<i>chpBf</i>	ATGGCTACGAGGTGGTGCTCAACG
<i>cphBr</i>	TCATGTTTCGACTCCTGTCTGGCGC
<b>Primers used for construction of ΔCHE4</b>	
<i>tlpFf</i>	TCCCTCGGTGTTTCGTTACCATCC
<i>cheY2r</i>	TGCGCGTCACTTCGAGCAGTTTCCG
<b>Primers used for construction of ΔCHE5</b>	
<i>tlpMf</i>	AAGATGTTGACGGTGGAAAAGGGC
<i>cheW3r</i>	AATGCCCTCGACCCGACCCATTGC
<i>cheB3f</i>	TCGAGGCAACTCGTCGGATCATGG
<i>cheY3r</i>	TTGGTCTCCAGCAACTGCTGCTGG
<b>Primers used for construction of TLCP1</b>	
<i>tlpCf</i>	GAAGGACTTCAACTCCTACGGCTCC
<i>tlpCr</i>	AAGCGACTGAAGCGTTTCTCCACG

pPT07.24, a 1.3-kb *SalI*-flanked *kan* cassette from pUC4K, and a 1.0-kb *SalI*-*Bam*HI fragment from pPT07.25 were ligated with the backbone of the *XbaI*/*Bam*HI-digested pUC118 to obtain pPT07.26.

#### 2.4.5. *Che* cluster 5

A 1.1-kb DNA fragment containing the 3' end of *tlpM* and the 5' end of *cheW*<sub>3</sub> was amplified with the primers *tlpM*f and *cheW*<sub>3</sub>r and the PCR product was cloned into pUC118 to make pPT07.27. A 1.4-kb DNA fragment containing the 3' end of *cheB*<sub>3</sub> and the 5' end of *cheY*<sub>3</sub> was amplified with the primers *cheB*<sub>3</sub>f and *cheY*<sub>3</sub>r and the PCR product was cloned into pUC118 to obtain pPT07.28. A 1.1-kb *KpnI*-*HincII* fragment from pPT07.27, a 1.3-kb *HincII* fragment containing *kan*, and a 1.3-kb *EcoRV*-*Bam*HI fragment from pPT07.28 were ligated with the backbone of the *KpnI*/*Bam*HI-digested pUC118 to obtain pPT07.29.

#### 2.4.6. *tlpC* (*aer*)

PCR was used to generate a fragment containing *tlpC*. The PCR product was cloned into pUC118 to give pTLPC01. pTLPC01 was partially digested with *Pst*I and ligated with a 1.3-kb *Pst*I-flanked *tet* gene cassette (conferring tetracycline resistance [Tc<sup>r</sup>]) from pUC118Tc to obtain pTLPC01.1.

#### 2.4.7. *tlpG* (*aer*-2)

A 2.1-kb *SalI* fragment containing *tlpG* was cloned into pUC118 to make pTLPG01. The *tlpG* gene was insertionally inactivated by cloning a 1.3-kb *SalI* flanked *kan* gene cassette into the *Xho*I site in the *tlpG* ORF on pTLPG01 to give pTLPG01.1.

The mutations were transferred into the chromosome of PAO1 by allelic exchange as described previously [19]. The deletion–insertions were confirmed by Southern hybridization with a digoxigenin non-radioactive DNA labeling and detection kit (Roche Diagnostics).

### 3. Results and discussion

#### 3.1. *Che* cluster 1 and *cheR* are required for aerotaxis

The aerotactic responses of *P. aeruginosa* were assessed with the chemotaxis well chamber method [8]. When both the upper and lower wells contained HEPES buffer alone, *P. aeruginosa* PAO1(pMRP9-1) moved from the lower to the upper well through the filter, responding to the gradient of oxygen. After the *gfp*-tagged *P. aeruginosa* PAO1 cells were introduced into the lower well, the GFP fluorescence intensity in the upper well continuously increased (Fig. 2A). The *gfp*-tagged  $\Delta$ *pilH**J**K* ( $\Delta$ *pilH pilI pilJ pilK*),  $\Delta$ *CHE3* ( $\Delta$ *pilJ pilK pilL chpA chpB*), and  $\Delta$ *CHE5* ( $\Delta$ *cheW*<sub>3</sub> *cheR*<sub>3</sub> *cheW*<sub>4</sub> *cheA*<sub>3</sub> *cheB*<sub>3</sub>) cells exhibited normal aerotactic responses, however,  $\Delta$ *CHE1* ( $\Delta$ *cheY cheZ cheA cheB motA*<sub>2</sub> *motB*<sub>2</sub> *cheW*)

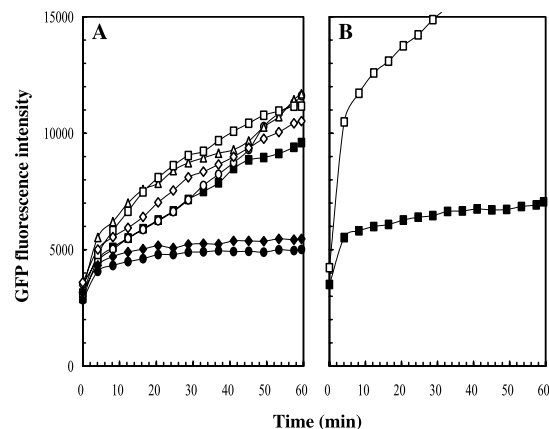


Fig. 2. Aerotactic responses by wild-type and mutant strains of *P. aeruginosa*. The changes in the GFP fluorescence intensity of the upper well were measured by a fluorescence spectrometer. (A) (○), PAO1 (wild-type); (●),  $\Delta$ *CHE1* ( $\Delta$ *cheY cheZ cheA cheB motA*<sub>2</sub> *motB*<sub>2</sub> *cheW*); (△),  $\Delta$ *CHE3* ( $\Delta$ *pilJ pilK pilL chpA chpB*); (□),  $\Delta$  *pilH**J**K* ( $\Delta$ *pilH pilI pilJ pilK*); (■),  $\Delta$ *CHE1*(pPT07.1); (◇),  $\Delta$ *CHE5* ( $\Delta$ *cheW*<sub>3</sub> *cheR*<sub>3</sub> *cheW*<sub>4</sub> *cheA*<sub>3</sub> *cheB*<sub>3</sub>); (◆), PC4 (*cheR* mutant). (B) (■),  $\Delta$ *CHE4* ( $\Delta$ *cheY*<sub>2</sub> *cheA*<sub>2</sub> *cheW*<sub>2</sub> *tlpG cheR*<sub>2</sub> *cheB*<sub>2</sub>); (□),  $\Delta$ *CHE4*(pTLPG01.2).

*cheB motA*<sub>2</sub> *motB*<sub>2</sub> *cheW*) and PC4 (*cheR* mutant) had an impaired ability to respond to oxygen. Microscopic analysis showed that  $\Delta$ *CHE1* and PC4 were fully motile.  $\Delta$ *CHE1* and PC4 were also defective in chemotaxis toward peptone and phosphate (data not shown). pPT07.1 (carrying the Che cluster 1) restored the ability of  $\Delta$ *CHE1* to respond to oxygen (Fig. 2A). These results suggest that the mutation phenotypes were not due to polar effects of the cassette insertion and the Che cluster 1 was required for aerotaxis in *P. aeruginosa* PAO1. The *cheR* gene was shown to be essential for aerotaxis, suggesting that aerotaxis is the MCP-dependent chemotaxis [25].

#### 3.2. *tlpG* is a chemotactic transducer for aerotaxis

$\Delta$ *CHE4* ( $\Delta$ *cheY*<sub>2</sub> *cheA*<sub>2</sub> *cheW*<sub>2</sub> *tlpG cheR*<sub>2</sub> *cheB*<sub>2</sub>) showed decreased aerotaxis (Fig. 2B).  $\Delta$ *CHE4* was fully motile. In contrast to  $\Delta$ *CHE1*,  $\Delta$ *CHE4* exhibited positive chemotactic responses to peptone and phosphate (data not shown) and the Che cluster 4 contains the *mcp*-like gene, *tlpG*. These findings suggest the possibility that TlpG is a chemotactic transducer for aerotaxis and the mutation phenotype of  $\Delta$ *CHE4* is due to the deletion of the *tlpG* gene. To investigate this possibility, pTLPG01.2 (containing *tlpG*) was introduced into  $\Delta$ *CHE4* and the transformant was examined for aerotaxis. The introduction of pTLPG01.2 restored the ability of  $\Delta$ *CHE4* to respond to oxygen (Fig. 2B). To confirm that TlpG is a chemotactic transducer for aerotaxis, we inactivated the *tlpG* gene by inserting a *kan* cassette into the wild-type gene in the PAO1 genome. The single *tlpG* mutant, designated TLPG1, showed decreased aerotaxis (Fig. 3A). pTLPG01.2 complemented the mutation of TLPG1, indicating the absence of polar effects.  $\Delta$ *CHE4*(pTLPG01.2) and TLPG1(pTLPG01.2) showed stronger aerotactic re-



sponses than PAO1 (Figs. 2B and 3A). PAO1(pTLPG01.2) also showed stronger aerotaxis than PAO1 (Fig. 3A). These results indicate that the level of TlpG protein in the cell determines the strength of its positive aerotactic response.

### 3.3. Identification of *TlpC* as a second aerotaxis transducer

Although *tlpG*-deficient cells showed decreased aerotactic responses, aerotaxis was not abolished in *tlpG*-deficient cells (Fig. 3A). This result suggests the existence of an additional transducer for aerotaxis. To identify an additional transducer for aerotaxis, a series of mutants that have deletion–insertion mutations in individual *mcp*-like genes in the PAO1 genome were constructed. PAO1 possesses 26 *mcp*-like genes [15–17]. We amplified 25 *mcp*-like genes other than *tlpG* by PCR using the sequence-specific primers, cloned into the vector plasmid pUC118, and disrupted individual genes by inserting a *tet* cassette into the wild-type genes in the PAO1 genome. After the introduction of pMRP9-1, each mutant was tested for aerotaxis. Aerotaxis assays revealed that the *tlpC* (PA1561) mutant TLPC1 showed decreased aerotaxis (Fig. 3B). pTLPC01.2 carrying the *tlpC* gene complemented the mutation of TLPC1. TlpC overproduction also increased the aerotactic response (PAO1[pTLPC01.2], Fig. 3B). We further constructed the double mutant TLPCG1 by inserting a *tet* cassette into the wild-type *tlpC* gene in the TLPG1 genome. Aerotaxis was abolished in the *tlpC tlpG* double mutant TLPCG1 (Fig. 3C). Aerotaxis was partially restored in the *tlpC tlpG* double mutant when TlpC or TlpG was expressed from a plasmid. These results suggest that *P. aeruginosa* PAO1 possesses two aerotaxis transducers, TlpC and TlpG.

### 3.4. Sequence analysis of the *tlpC* and *tlpG* genes

The potential product of *tlpC* was 521-amino acid TlpC.

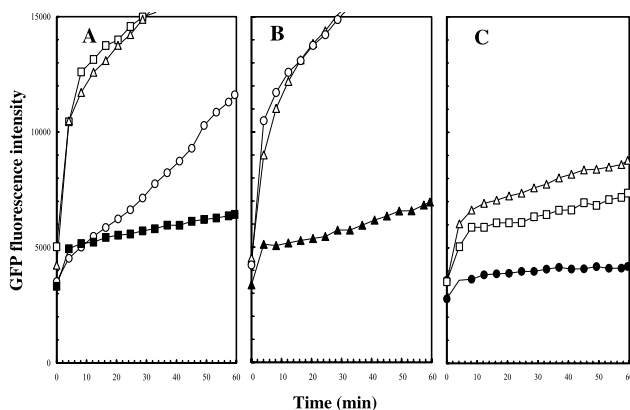


Fig. 3. Aerotactic responses by *P. aeruginosa* strains. (A) ○, PAO1; ■, TLPG1 ( $\Delta tlpG$ ); □, TLPG1(pTLPG01.2); △, PAO1(pTLPG01.2). (B) ▲, TLPC1 ( $\Delta tlpC$ ); ○, TLPC1(pTLPC01.2); △, PAO1(pTLPC01.2). (C) ●, TLPCG1 ( $\Delta tlpC tlpG$ ); △, TLPCG1(pTLPG01.2); □, TLPCG1(pTLPC01.2).

The *tlpC* gene product is 78 and 45%, respectively, identical to the *Pseudomonas putida* and *E. coli* Aer proteins, transducers involved in aerotaxis [11,12,26]. The N-terminal domain of the *tlpC* gene product (residues 21–106) contains a PAS motif, which is known to comprise a binding pocket for a prosthetic group [14]. Only one hydrophobic sequence (residues 146–191) was predicted in the TlpC protein. This hydrophobic sequence may serve to anchor TlpC to the cytoplasmic side of the inner membrane. TlpC residues 354–397 are 84% identical to the 44-amino acid highly conserved domain (HCD) of the *E. coli* chemotaxis transducer Tsr. MCPs from phylogenetically diverse bacteria have been shown to possess the HCD [27], which is likely to be important for the interaction between MCPs and CheW as well as CheA [28]. Thus the TlpC protein closely resembled the *P. putida* and *E. coli* Aer proteins and it was experimentally demonstrated that the *tlpC* gene is involved in aerotaxis. On this basis, the *tlpC* gene was renamed *aer*.

The putative product of the *tlpG* gene was a 679-residue protein (72.6 kDa) that had a predicted C-terminal segment (residues 481–524) with 89% identity to the HCD of the *E. coli* Tsr protein. In the C-terminal domain of TlpG, there are two potential methylation regions (residues 412–434 and 600–624) that have 87 and 72% identity to the K1 and R1 regions of *E. coli* Tsr [29], respectively. Thus, the C-terminal domain of TlpG has typical structural features of MCPs. A hydropathy plot of TlpG indicated that two hydrophobic membrane-spanning regions in the N-terminal domain of typical MCPs are missing in TlpG. TlpG may be a cytoplasmic protein and sense intracellular stimuli. Reverse position-specific Blast search [30] against the database of the National Center for Biotechnology Information predicted the presence of a PAS motif in the N-terminal domain of TlpG (residues 174–216). TlpC and TlpG had 18% identity in the PAS domain. Since genetic analysis demonstrated the involvement of the *tlpG* gene in aerotaxis, *tlpG* was renamed *aer-2*. No significant homology other than the PAS motif was detected in the N-terminal domains between Aer and Aer-2.

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