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Identification of Chemosensory Proteins for Trichloroethylene in *Pseudomonas aeruginosa*

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The involvement of the chemotaxis gene cluster 1 (*cheYZABW*) and *cheR* in repellent responses of *Pseudomonas aeruginosa* to trichloroethylene (TCE) is described and three methyl-accepting chemotaxis proteins (MCPs) for TCE are identified. TCE chemotaxis assays of a number of deletion-insertion mutants of *P. aeruginosa* PAO1 revealed that the chemotaxis gene cluster 1 and *cheR* are required for negative chemotaxis to TCE. Mutant strains which contained deletions in *pctA*, *pctB* and *pctC* showed decreased responses to TCE. The *pctA*, *pctB* and *pctC* genes have been reported to encode MCPs for amino acids [K. Taguchi *et al*., Microbiology, 143, 3223-3229, (2000)]. The *pctA* mutation more severely impaired chemotactic responses to TCE than did those of *pctB* and *pctC*, suggesting that PctA is the major MCP for TCE among the three MCPs. The *pctA*, *pctB* and *pctC* mutant strains showed decreased responses to chloroform and methylthiocyanate. This result demonstrates that PctA, PctB and PctC are also involved in repellent responses to chloroform and methylthiocyanate.

[**Key words:** chemotaxis, chemosensory protein, trichloroethylene, methyl-accepting chemotaxis protein, *Pseudomonas aeruginosa*]

Chemotaxis is the movement of an organism toward chemical attractants and away from chemical repellents (1). *Pseudomonas aeruginosa* is an obligately aerobic bacterium and is capable of swimming by rotating a single polar flagellum. This bacterium, like most other motile bacteria, exhibits chemotactic responses to a wide range of chemical stimuli. *P. aeruginosa* is attracted to 20 commonly occurring amino acids (2, 3), sugars (4), organic acids (5) and inorganic phosphate (6). It has repellent responses (negative chemotaxis) to thiocyanate and isothiocyanate esters (7).

The molecular mechanisms that underlie bacterial chemotaxis have been studied intensively with *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (8). Chemotactic ligands are detected by cell surface chemoreceptors called methyl-accepting chemotaxis proteins (MCPs). Upon binding a chemotactic ligand, a MCP generates chemotaxis signals that are communicated to the flagellar motor via a series of chemotaxis (Che) proteins. *E. coli* possesses 5 MCPs and 6 Che proteins. Analysis of the complete genome sequence of *P. aeruginosa* PAO1 suggested that the *P. aeruginosa* chemosensory system is very complex, with more than 20 *che* genes in five distinct clusters and 26 *mcp*-like genes scattered throughout the genome (6, 9, 10). We demonstrated that *cheY*, *cheZ*, *cheA*, *cheB* and *cheR* in Che cluster 1 and *cheR* in Che cluster 2 are responsible for positive chemotactic responses to attractants (11-13). We also found that seven *mcp* genes encode MCPs for amino acids (*pctA*, *pctB* and *pctC*) (3, 14), inorganic phosphate (*ctpH* and *ctpL*) (6) and O_2 (*aer* and *aer-2*) (13). However, the mechanisms of negative chemotaxis in *P. aeruginosa* are still poorly understood.

Chloroethylenes are the most frequently detected groundwater contaminants (15). Widespread environmental contamination by chloroethylenes is of concern due to the toxicity and carcinogenicity of these compounds. In a recent study, we demonstrated that volatile chlorinated aliphatic compounds including chloroethylenes and chloroform were toxic to microorganisms (16). We also found that *P. aeruginosa* was repelled by trichloroethylene (TCE) and chloroform (16). In this study, we genetically analyzed negative chemotaxis to TCE in *P. aeruginosa* and found that TCE was detected by PctA, PctB and PctC, which are MCPs for amino acids. We also present evidence that these MCPs are involved in repellent responses to chloroform and methylthiocyanate in *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains and plasmids The bacterial strains and plasmids used in this present study are shown in Table 1. *E. coli* MV1184, which was used for plasmid construction and DNA manipulation, was grown at 37˚C with shaking in 2 x YT medium (22) supplemented with appropriate antibiotics. This medium was also used for the *P. aeruginosa* cells for chemotaxis and electroporation. *Pseudomonas putida* F1 was grown at 28˚C with shaking in LB medium (22).

TABLE 1. Bacterial Strains and Plasmids Used in This Study

Strain/plasmid	Relevant characteristics[a]	Source or reference
Strains		
E. coli		
MV1184	*ara* Δ(*lac-proAB*) *rpsL thi* (ϕ80 *lacZ*ΔM15) Δ(*srl-recA*)*306*::Tn*10*(Tc^r) F'[*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	17
P. aeruginosa		
PAO1	Prototroph, FP$^-$ (sex factor minus)	18
ΔCHE1	PAO1 derivative, ΔChe cluster 1 (*cheY cheZ cheA cheB* PA1460-1463[b] *cheW*)	13
ΔCHE3	PAO1 derivative, ΔChe cluster 3 (*pilJ pilK pilL chepA chpB*)	13
ΔCHE4	PAO1 derivative, ΔChe cluster 4 (*cheY2 cheA2 cheW2 aer-2 cheR2* PA0174[b] *cheB2*)	13
ΔCHE5	PAO1 derivative, ΔChe cluster 5 (*cheW3 cheR3 cheW4 cheA3 cheB3*)	13
PC4	NTG derived mutant of PAO1, *cheR*	12
PCTA1	PAO1 derivative, *pctA*::*kan*	14
PCTB1	PAO1 derivative, *pctB*::*kan*	14
PCTC1	PAO1 derivative, *pctC*::*kan*	14
PCT2	PAO1 derivative, Δ(*pctA pctB pctC*)	14
P. putida		
F1	Toluene-oxidizing bacterium	19
Plasmids		
pUC118	General cloning vector; Ap^r	17
pUC4K	pUC4 containing a 1.3-kb *kan* gene cassette; Ap^r, Km^r	Amersham
pGEM-T Easy	PCR cloning vector; Ap^r	Promega
pUCP18	Broad-host-range cloning vector; Cb^r	20
pQF50	Broad-host-range transcriptional fusion vector; Cb^r *lacZ*	21
pMAI18-1	pUCP18 with a 2.1-kb PCR fragment containing *pctA*; Cb^r	This study
pMAI18-2	pUCP18 with a 2.1-kb PCR fragment containing *pctB*; Cb^r	This study
pMAI18-3	pUCP18 with a 2.1-kb PCR fragment containing *pctC*; Cb^r	This study
pMAI18-4	pUCP18 containing *pctA* under the control of the modified *pctA* promoter; Cb^r	This study
pMAI18-5	pUCP18 containing *pctB* under the control of the modified *pctA* promoter; Cb^r	This study
pMAI18-6	pUCP18 containing *pctC* under the control of the modified *pctA* promoter; Cb^r	This study
pMAI18-7	pUCP18 with a 2.1-kb PCR fragment containing the *pctA*-like gene from *P. putida* F1	This study
pMAI50-1	pQF50 with a 0.7-kb fragment containing	This study

	the promoter region of *pctA*; Cb^r *pctA*::*lacZ*	
pMAI50-2	pQF50 with a 0.7-kb fragment containing the promoter region of *pctB*; Cb^r *pctB*::*lacZ*	This study
pMAI50-3	pQF50 with a 0.7-kb fragment containing the promoter region of *pctC*; Cb^r *pctC*::*lacZ*	This study

[a] Ap, Ampicillin; Km, kanamycin; Cb, carbenicillin; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

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

Chemotaxis assays Computer-assisted capillary assays were carried out as described previously (23) and cells were videotaped over the first 2 min. Digital image processing was used to count the number of bacteria around the mouth of a capillary containing a known concentration of a test compound plus 1% agarose. The chemotaxis buffer used was 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.0).

DNA manipulation and electroporation Standard procedures were used for plasmid DNA preparations, restriction enzyme digestions, ligations, transformations and agarose gel electrophoresis (22). Polymerase chain reactions (PCRs) were carried out using KOD plus (Toyobo, Tokyo) DNA polymerase according to the manufacturer's instructions. Before cloning, PCR products were electrophoresed and purified from the 1% agarose gel using the GenecleanII kit (Bio101, Carlsbad, CA, USA). *P. aeruginosa* was transformed by electroporation as described previously (11).

Plasmid construction Recombinant plasmids were constructed as detailed below. Genetic organization of the *pctA*, *pctB* and *pctC* genes and subclones are shown in Fig. 1. Oligonucleotides used for PCR are listed in Table 2.

The *pctA*, *pctB* and *pctC* genes in the *P. aeruginosa* PAO1 genome are found at genomic loci PA4309, PA4310 and PA07, respectively (http://www.pseudomonas.com). These genes, as well as flanking upstream DNA and downstream DNA, were amplified from the genomic DNA of *P. aeruginosa* PAO1 with oligonucleotides pctA1f versus pctA1r, pctB1f versus pctB1r and pctC1f versus pctC1r, respectively. The agarose-gel purified fragments were cloned into pUCD18, creating pMAI18-1, pMAI18-2 and pMAI18-3.

A 0.7-kb fragment of *P. aeruginosa* PAO1, corresponding to the region immediately upstream of the *pctA* open reading frame (ORF), was amplified by PCR (oligonucleotides, PropctAf and PropctAr) such that an *Nde*I site was introduced at the downstream end. By introduction of the *Nde*I site, the nucleotide sequence immediately upstream of the start codon of *pctA* was changed from AACATG to CATATG (the start codon is underlined). The PCR product was cloned into pUCP18, yielding pMAI18-PpctA. The *pctA*, *pctB* and *pctC* ORFs were amplified from pMAI18-1, pMAI18-2 and pMAI18-3 with oligonucleotides pctA2f versus pctA2r, pctB2f versus pctB2r and pctC2f versus pctCr2, respectively, such that an *Nde*I site was introduced at the upstream end. The PCR products were cloned into pUC118 to construct pMAI118-pctA2, pMAI118-pctB2 and pMAI118-pctC, respectively. The 2.0-kb *Nde*I-*Hin*dIII fragments of pMAI118-pctA2,

pMAI118-pctB2 and pMAI118-pctC2 were cloned between the *Nde*I and *Hin*dIII sites of pMAI18-PpctA to obtain pMAI18-4, pMAI18-5 and pMAI18-6, respectively.

Plasmid pQF50 is a broad-host-range transcriptional fusion vector (21), and contains a multicloning site upstream of the promoterless *lacZ* gene. The promoter regions of *pctA*, *pctB* and *pctC* were amplified by PCR with oligonucleotides pctA3f versus pctA3r, pctB3f versus pctB3r and pctC3f versus pctC3r such that an *Sph*I site and a *Bam*HI site were introduced at the upstream and downstream ends, respectively. The PCR products were treated with *Sph*I and *Bam*HI and cloned between the *Sph*I and *Bam*HI sites of pQF50 to construct pMAI50-1, pMAI50-2 and pMAI50-3, respectively.

A 2.1-kb DNA fragment containing the *pctA*-like gene was amplified from the *P. putida* F1 genome with the PCR forward primer pctAPpf and reverse primer pctAPpr. The PCR product was cloned into pUCD18 to give pMAI18-7.

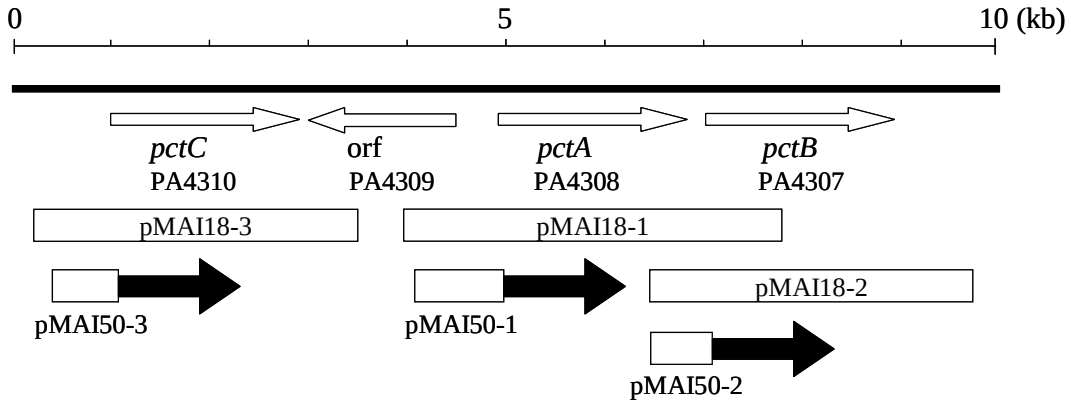

FIG. 1. Genetic organization of the *pctC pctA pctB* region of *P. aeruginosa* and DNA fragments used for plasmid construction. The locations and orientations of individual ORFs are shown by open horizontal arrows. Gene ID numbers used in the *P. aeruginosa* genome sequencing project (http://www.pseudomonas.com) are indicated below gene names. Open bars indicate DNA fragments subcloned into pUCD18 or pQF50. Black arrows are the promoterless *lacZ* gene in pQF50.

TABLE 2. Oligonucleotides Used for PCR

Primer	Sequence (5’ to 3’)
pctA1f	CTCGCCAAGGATTTCCGGATTGTCC
pctA1r	TTGACCGGAGAGAAACTGAGGATCC
pctA2f	GCATATGATCAAAAGTCTGAAGTTCAGCC
pctA2r	TTGACCGGAGAGAAACTGAGGATCC
pctA3f	CGCATGCCTATCGGTCAACTCCAGGAGGTTGG
pctA3r	TGGATCCTGTAGAGGGTGAACAGTGCGAAAGC
pctB1f	AGATGATCGAAGAGCTGCAAGTCGG

pctB1r	CATCAGCTTTTCCGCCATTACCCGG
pctB2f	CCATATGCAAGAAACCATGATCAAAAGTC
pctB2r	CATCAGCTTTTCCGCCATTACCCGG
pctB3f	CGCATGCCCCAGCAGATCCAGAAGATGATCGA
pctB3r	TGGATCCTTGTAGAGGGTGAACAGGGAGAAGG
pctC1f	AGGTCGATGAACGGAAAATCACCGG
pctC1r	CTTCACCGCCAAGGAGCAGAAATCG
pctC2f	CCATATGCTTCGCTCGCTGTCGTTTGCC
pctC2r	CTTCACCGCCAAGGAGCAGAAATCG
pctC3f	CGCATGCCGATAGGCCTGAATCTAGACTCGGG
pctC3r	GGGATCCGTAGAGGATGAAGCAGCTGAAGGCG
PropctAf	CTCGCCAAGGATTTCCGGATTGTCC
PropctAr	GATCATATGGTATTGCGTCCAGGAGTATG
pctAPpf	TGTTGCACAACCTACGGCTCCACTCG
pctAPpr	CGAATGTGTAGCCTCTATCAGATGC

β-Galactosidase assay For β-galactosidase measurements, *P. aeruginosa* was grown with shaking in 2x YT at 37˚C. β-Galactosidase activities of *P. aeruginosa* cells were determined as described by Miller (24), with the modification that the enzymatic reaction was carried out at 37˚C.

Chemicals All chemicals were reagent grade. Trichloroethylene was purchased from Wako Pure Chemical Industries, Osaka. Methylthiocyanate was obtained from Tokyo Kasei Kogyo, Tokyo. All other chemicals were obtained from Nacalai Tesque, Kyoto.

RESULTS

***che* genes responsible for chemotactic responses to TCE** The chemotactic responses to TCE by *P. aeruginosa* were assayed using the computer-assisted capillary method. This method is a reproducible and quantitative method for analyzing bacterial behavioral responses to chemical stimuli (23). Figure 2 shows the time-course data on the negative chemotactic responses of *P. aeruginosa* PAO1 toward TCE. The movement of bacterial cells was so dramatic that the bacterial numbers around the mouth of the capillary containing 500 mg l^{-1} of TCE plus 1% agarose decreased by approximately 60% within 60 s after the start of microscopic observation.

P. aeruginosa PAO1 possesses more than 20 *che* genes situated in five clusters (6, 9, 10). To investigate which Che cluster(s) is responsible for negative chemotaxis to TCE, Che cluster mutants were examined for their responses to TCE. ΔCHE1, the mutant strain of PAO1 with a deletion of Che cluster 1 (*cheY, cheZ, cheA, cheB, motA*$_2$*, motB*$_2$ and *cheW*), and PC4 (*cheR* mutant) were fully motile, but did not move away from TCE (Fig. 2A). Che cluster 3 deletion mutant ΔCHE3 (Δ*pilH pilI pilJ pilK chpA chpB*) Che cluster 4

mutant ΔCHE4 (Δ*cheY2 cheA2 cheW2 aer cheR2 cheB2*), and Che cluster 5 mutant ΔCHE5 (Δ*cheW3 cheR3 cheW4 cheA3 cheB3*) showed normal responses to TCE (data not shown). These results indicate that, as is the case with positive chemotaxis, Che cluster 1 and *cheR* are essential for negative chemotaxis to TCE. The *cheR* gene was shown to be essential for chemotactic responses to TCE, suggesting that negative chemotaxis to TCE is the MCP-dependent chemotaxis (25).

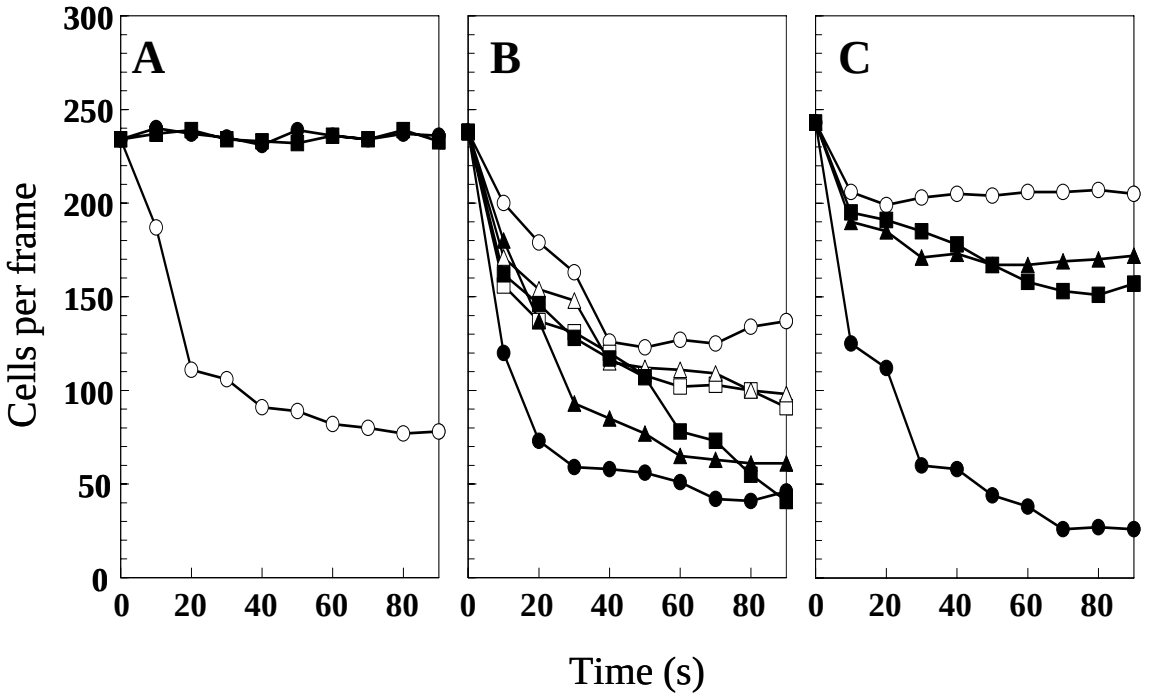

FIG. 2. Chemotactic responses to TCE by *P. aeruginosa* strains. Digital image processing was used to count the number of bacteria around the mouth of a capillary containing 500 mg/*l* of TCE and 1% agarose. One videotape frame was analyzed at each time point. The chemotactic response is presented as the number of bacteria per videotape frame as described previously (23). (A) Wild-type PAO1 (open circles), ΔCHE1 (the Che cluster 1 deletion mutant) (closed circles) and PC4 (the *cheR* mutant) (closed squares). (B) PCTA1 (*pctA*::*kan*) (open circles), PCTB1 (*pctB*::*kan*) (open squares), PCTC1 (*pctC*::*kan*) (open triangles), PCTA1(pMAI18-1) (closed circles), PCTB1(pMAI18-2) (closed squares) and PCTC1(pMAI18-3) (closed triangles). (C) PCT2 (the *pctA pctB pctC* triple mutant) (open circles), PCT2(pMAI18-1) (closed circles), PCT2(pMAI18-2) (closed squares) and PCT2(pMAI18-3) (closed triangles).

PctA, PctB and PctC are chemotactic transducers for negative chemotaxis to TCE *P. aeruginosa* has 26 *mcp*-like genes in its genome (6, 9, 10). In previous studies, we constructed a series of mutants that have deletion-insertion mutations in individual *mcp*-like genes in the PAO1 genome (6, 13, 14). To identify a MCP(s) for negative chemotaxis to TCE, we tested each mutant for chemotaxis to TCE. Chemotaxis

assays revealed that *pctA* mutant PCTA1, *pctB* mutant PCTB1 and *pctC* mutant PCTC1 showed decreased chemotactic responses to TCE (Fig. 2B). pMAI18-1 (carrying *pctA*), pMAI18-2 (carrying *pctB*) and pMAI18-3 (carrying *pctC*) complemented the mutations of PCTA1, PCTB1 and PCTC1, respectively, showing that the mutation phenotypes were not due to polar effects of the kanamycin resistant gene cassette insertions (Fig. 2B). Although the *pctA pctB pctC* triple mutant PCT2 showed weaker responses to TCE than the single mutants, negative chemotaxis to TCE was not abolished in PCT2 (Fig. 2C). This result suggests the existence of an additional transducer for TCE. However, significant decreased responses to TCE were not detected for other *mcp* mutants. These results suggest that although there are some MCPs involved in chemotxis to TCE other than PstA, PctB and PctC, their contributions to TCE chemotaxis are much smaller than those of PctA, PctB and PctC. Therefore, further investigations were focused on *pctA*, *pctB* and *pctC*.

PctA is the major transducer for TCE The *pctA* mutation more severely impaired chemotactic responses to TCE than did the *pctB* and *pctC* mutations (Fig. 2B). This could mean that the contribution of PctA to TCE chemotaxis is greater than those of PctB and PctC. In fact, this is consistent with the result of the complementation test of the *pctA pctB pctC* triple mutant PCT2 by pMAI18-1, pMAI18-2 and pMAI18-3. pMAI18-2 (carrying *pctB*) and pMAI18-3 (carrying *pctC*) partially restored the ability of PCT2 to respond to TCE, whereas PCT2 harboring pMAI18-1 (carrying *pctA*) showed stronger responses to TCE than did the wild-type strain PAO1 (Fig. 2C). Thus, PctA is the predominant chemotaxis transducer for TCE among PctA, PctB and PctC. This may be due to differences in expression levels of these MCPs. Alternatively, it could be that one molecule of PctA generates stronger chemotactic signals than one molecule of PctB or PctC in response to the same level of TCE stimulus.

To assess the possibilities, the promoter regions of *pctA*, *pctB* and *pctC* were inserted individually upstream of the promoterless *lacZ* gene in the transcriptional fusion vector pQF50. PAO1 was transformed with either pMAI50-1 (carrying *pctA*::*lacZ*), pMAI50-2 (carrying *pctB*::*lacZ*) or pMAI50-3 (carrying *pctC*::*lacZ*) and β-galactosidase activities were measured in transformant strains (Table 3). The β-galactosidase levels were approximately 2-10-fold higher in PAO1(pMAI50-2) than in PAO1(pMAI50-1) and PAO1(pMAI50-3), suggesting that the promoter activity of *pctA* is not the highest among those of the three genes. To investigate the second possibility, we constructed the *pctA* promoter-based expression vector pMAI18-PpctA and subcloned the *pctA*, *pctB* and *pctC* ORFs into it to obtain pMAI18-4, pMAI18-5 and pMAI18-6, respectively. The 0.7-kb regions immediately upstream of the start codons of the *pctA*, *pctB* and *pctC* ORFs are identical in pMAI18-4, pMAI18-5 and pMAI18-6 and expression levels of PctA, PctB and PctC in transformant strains were expected to be almost the same. The resulting plasmids were introduced into the *pctA pctB pctC* triple mutant PCT2 and transformant strains were examined for TCE chemotaxis. PCT2 harboring pMAI18-4 (carrying *pctA*) showed stronger responses to TCE than did the wild-type strain PAO1, however, pMAI18-5 (carrying *pctB*) and pMAI18-6 (carrying *pctC*) partially

restored the ability of PCT2 to respond to TCE (data not shown). These results suggest that PctA is the predominant MCP among the three MCPs because chemotactic signals generated by PctA are stronger than those of PctB and PctC.

TABLE 3. β-Galactosidase Activities in *P. aeruginosa* Strains

Strain	Relevant characteristics	β-Galactosidase activity [a] (Miller unit)
PAO1(pQF50)	Control	11±1
PAO1(pMAI50-1)	*pctA*::*lacZ*	145±2
PAO1(pMAI50-2)	*pctB*::*lacZ*	337±9
PAO1(pMAI50-3)	*pctC*::*lacZ*	33±1

[a] Values are the means ± the standard deviations of four separate assays.

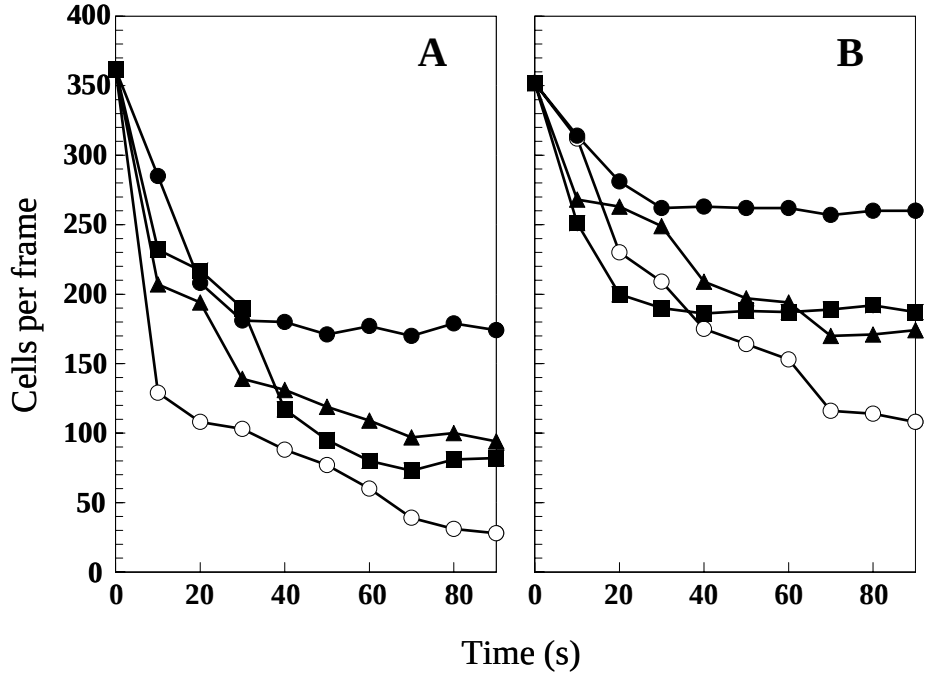

FIG. 3. Chemotactic responses of PAO1 (open circles), PCTA1 (closed circles), PCTB1 (closed squares) and PCTC1 (closed triangles) for chloroform (A) and methylthiocyanate (B). The chloroform and methylthiocyanate concentrations in the capillary were 15 mg/*l* and 100 mg/*l*, respectively.

PctA, PctB and PctC are involved in negative chemotaxis to chloroform and methylthiocyanate *P. aeruginosa* is repelled by chloroform and thiocyanate esters including methylthiocyanate (7, 16). To investigate whether PctA, PctB and PctC are involved in chemotaxis to these repellents, mutant strains were examined for chemotactic responses towards chloroform and methylthiocyanate. As shown in Fig. 3,

the wild-type strain PAO1 was strongly repelled by chloroform and methylthiocyanate. The *pctA*, *pctB* and *pctC* single mutants, PCTA1, PCTB1 and PCTC1, showed decreased responses to both chloroform and methylthiocyanate. As was the case with TCE, the *pctA* mutation more severely impaired chloroform and methylthiocyanate chemotaxis. Thus, it was demonstrated that PctA, PctB and PctC are also MCPs for chloroform and methylthiocyanate and PctA serves as the major chemoreceptor among the three MCPs.

DISCUSSION

In previous studies, we demonstrated that the *pctA*, *pctB* and *pctC* genes, which are clustered on the *P. aeruginosa* PAO1 genome (Fig. 1), encode MCPs for amino acids (3, 14). Genetic analysis revealed that PctB and PctC respond to seven L-amino acids (alanine, arginine, glutamate, glutamine, lysine, methionine and tyrosine) and two L-amino acids (histidine and proline), while PctA detects 18 L-amino acids (all naturally occurring L-amino acids except glutamine and aspartate). In this study, it was shown that these amino acid chemoreceptors also mediate the repellent responses to TCE, chloroform and methylthiocyanate. The property whereby one MCP responds to both attractants and repellents is not uncommon. For example, *E. coli* Tsr perceives both attractants (serine, alanine and glycine) and repellents (acetate, leucine, benzoate and indole) (26).

PctA, PctB and PctC exhibit typical structural features of MCPs (27): a positively charged N-terminus followed by a hydrophobic membrane-spanning region, a hydrophilic periplasmic domain, a second hydrophobic membrane-spanning region and a hydrophilic cytoplasmic domain. Genetic analysis of *E. coli* and *S. enterica* serovar Typhimurium MCPs revealed that chemotactic ligands bind to periplasmic domains of MCPs and their bindings initiate chemotaxis signal transduction (25). The diverse ligand specificities among MCPs reflect amino acid sequence diversities of periplasmic domains of MCPs. There are high degrees of similarity (50-70% identities) in the potential periplasmic domains among PctA, PctB and PctC. These regions have no significant similarity to any other *P. aeruginosa* MCPs. Therefore, it is not an unreasonable result that these three MCPs were identified as chemotactic transducers for the same set of repellents.

Parales *et al*. (28) and Vardar *et al*. (29) investigated the chemotaxis of the TCE-degrading bacterium *P. putida* F1 to TCE and reported that this bacterium was attracted by TCE. They assayed chemotactic responses by the agarose plug assay, in which an agarose plug containing test compounds was placed in bacterial cell suspensions and positive chemotactic responses were detected as the formation of chemotactic rings around the agarose plug. *P. putida* F1 formed chemotactic rings around agarose plugs with TCE. Although bacterial cells accumulated near the plugs, they did not touch them. These results suggest the possibility that *P. putida* F1 possess both chemoreceptors for the positive response and for the repellent response to TCE and consequently, cells accumulate to an optimal concentration of TCE. Since the genome

of *P. putida* KT2440 has already been sequenced (30), we searched for the *pctA* homologue of *P. putida*. A blast search of the KT2440 genome sequence found that the gene at the genomic locus PP1371 (http://www.tigr.org) encodes a polypeptide that has 68% identity to PctA (58% identity in the potential periplasmic domains). It is possible that *P. putida* F1 possesses a *pctA*-like gene and the gene product is involved in the repellent response to TCE. PCR with oligonucleotides pctAPpf and pctAPpr amplified a 2.1-kb product from the *P. putida* F1 genome and sequence analysis of this PCR product revealed that *P. putida* F1 possesses a gene almost identical to PP1371. pMAI18-7 (carrying the *P. putida* F1 *pctA*-like gene) restored the ability of the *pctA pctB pctC* triple mutant PCT2 to respond to TCE (Fig. 4). Thus, the *pctA*-like gene could be involved in the negative taxis to TCE in *P. putida* F1. Bacterial chemotaxis is likely to be of importance for strategies aimed at using bacteria to detoxify polluted environments as it can overcome some of the limitations of *in situ* bioremediation such as poor bioavailability due to mass transfer limitations, low solubility or sequestration of a chemical to a matrix surface (29, 31, 32). The inactivation of the *pctA*-like gene may enable *P. putida* F1 to accumulate to higher concentrations of TCE. We are currently investigating the possibility and are identifying the genes responsible for the positive taxis to TCE in *P. putida* F1. Further work is also being carried out in our laboratory to elucidate how pctA, PctB and PctC recognize such diverse ligands.

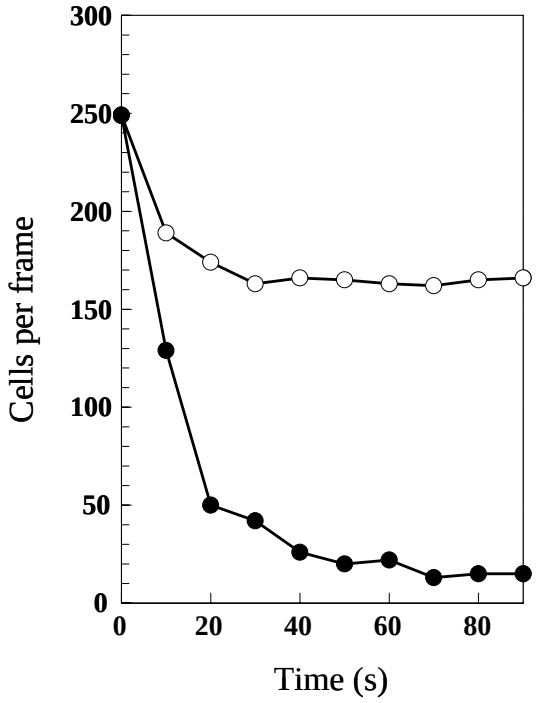

FIG. 4. Chemotactic responses of PCT2 (open circles) and PCT2(pMAI18-7) (closed circles) to TCE.

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