JOURNAL OF BACTERIOLOGY, Sept. 2006, p. 6700–6702 Vol. 188, No. 18
0021-9193/06/$08.00+0 doi:10.1128/JB.00584-06

Identification and Characterization of the Chemotactic Transducer in *Pseudomonas aeruginosa* PAO1 for Positive Chemotaxis to Trichloroethylene

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Pseudomonas aeruginosa **PAO1 is repelled by trichloroethylene (TCE), and the methyl-accepting chemotaxis proteins PctA, PctB, and PctC serve as the major chemoreceptors for negative chemotaxis to TCE. In this study, we found that the *pctABC* triple mutant of *P. aeruginosa* PAO1 was attracted by TCE. Chemotaxis assays of a set of mutants containing deletions in 26 potential *mcp* genes revealed that *mcpA* (PA0180) is the chemoreceptor for positive chemotaxis to TCE. McpA also detects tetrachloroethylene and dichloroethylene isomers as attractants.**

Chloroethylenes, such as trichloroethylene (TCE), are the most frequently detected groundwater contaminants (14). Widespread environmental contamination by chloroethylenes is of concern due to their toxicity and carcinogenicity. Although several bacteria are known to cometabolically degrade TCE, until now organisms capable of growing on TCE as the sole source of carbon and energy have not been found. Some bacteria, however, are attracted by TCE. For example, Parales et al. (10) and Varder et al. (18) demonstrated that the TCE-degrading bacteria *Pseudomonas putida* F1, *Burkholderia cepacia* G4, and *Pseudomonas stutzeri* OX1 are attracted by TCE. These TCE-degrading bacteria have been intensively studied as bioremediation agents in chloroethylene-polluted environments. The migration of TCE-degrading bacteria toward TCE might speed up the biodegradation process because it should bring the cells into contact with TCE (11). Despite these findings, very little is known about the chemosensory protein mediating positive chemotaxis to TCE. In this study, we report attractive responses to TCE by a mutant strain of *Pseudomonas aeruginosa* PAO1 and identification of a methyl-accepting chemotaxis protein (MCP) for positive chemotaxis to TCE.

In a previous study, we found that *P. aeruginosa* PAO1 (5) is repelled by TCE (15). Genetic analysis revealed that the MCPs PctA, PctB, and PctC, which were identified as MCPs for amino acids (7, 17), serve as the major chemoreceptors for the negative chemotaxis to TCE (16). In the previous study, we used the computer-assisted capillary method for chemotaxis assay (9). Parales et al. (10) and Varder et al. (18) found chemotactic responses toward TCE by TCE-degrading bacteria by the agarose plug method. Therefore, we first reexamined the wild-type PAO1 and the *pctABC* triple mutant PCT2 (17) for chemotactic responses to TCE by the agarose plug method. Agarose plug assays were carried out as previously described (20) with modifications. Molten 3% (wt/vol) agarose (Agarose S; Nippon Gene Inc., Toyama, Japan) in chemotaxis buffer (10 mM HEPES, pH 7.0) was kept at 60°C. The agarose was mixed with the same volume of chemotaxis buffer containing a known concentration of a test compound. Immediately after being mixed, 12 μl of the mixture was placed on a microscope slide, and a coverslip supported by two staples (0.5 mm in diameter) was placed on top to form a chamber. Cells were harvested in the early stationary phase and resuspended in chemotaxis buffer to a turbidity at 600 nm of approximately 1, and 120 μl of cell suspension was pipetted between the microscope slide and the coverslip. Consistent with results from the computer-assisted capillary method, PAO1 cells were repelled by TCE and formed clear zones around agarose plugs containing 4 mM TCE (Fig. 1A). The *pctABC* mutant PCT2 formed smaller clear zones than the wild-type PAO1. We then tested these strains for chemotactic responses to a lower concentration (1 mM) of TCE. As expected, PAO1 formed smaller clear zones around agarose plugs containing 1 mM TCE. Unexpectedly, however, PCT2 cells were attracted by agarose plugs containing 1 mM TCE and formed dense zones around the plugs. The attractive responses to TCE by PCT2 were reproducible. These results suggest that *P. aeruginosa* PAO1 has the ability to both negatively and positively respond to TCE. The negative responses are strong compared to the positive responses; thus, the positive responses were detected only after disruption of the *pctABC* genes encoding the major MCPs for negative chemotaxis to TCE.

P. aeruginosa PAO1 possesses 26 potential *mcp* genes in its genome (2, 19). We previously constructed a series of mutants that have deletion-insertion mutations in individual *mcp*-like genes in the PAO1 genome (6, 17, 19). To identify the MCP mediating positive chemotaxis to TCE, we tested each mutant by the agarose plug method. We noticed that the *mcpA* (gene identification number PA0180 in the *P. aeruginosa* genome sequencing project [http://www.pseudomonas.com/]) mutant PAO-dF formed slightly larger and clearer repellent zones around the agarose plugs than did the wild-type PAO1 (Fig. 1B). This result suggests that McpA is an MCP for positive TCE chemotaxis. To investigate whether McpA functions as an MCP for TCE, the *mcpA* gene was cloned into the broad-host-range vector pUCP18 (13) and the resulting plasmid, pHEK01,

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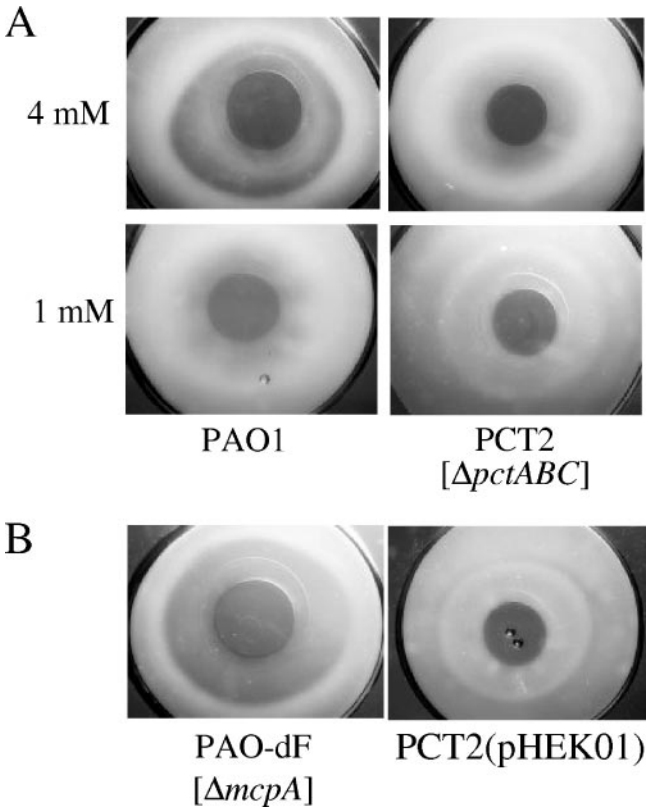

FIG. 1. Chemotactic responses of *P. aeruginosa* strains to TCE in agarose plug assays. Photographs were taken 10 min after the addition of *P. aeruginosa* strains. (A) Responses of the wild-type *P. aeruginosa* PAO1 and the *pctABC* mutant PCT2 to 1 mM and 4 mM TCE. (B) Responses of the *mcpA* mutant PAO-dF and PCT2, harboring pHEK01 (carrying *mcpA*), to 4 mM TCE. *P. aeruginosa* cells grown at 37°C in T_2 minimal medium containing glucose as the sole source of carbon (19) were used for these assays.

was introduced into PCT2. Because PCT2 exhibits decreased negative chemotaxis to TCE, it was expected that PCT2 harboring pHEK01 would show stronger positive chemotaxis to TCE. In fact, as expected, PCT2(pHEK01) cells were attracted by even 4 mM TCE (Fig. 1B). To further confirm the result, we investigated whether the *P. aeruginosa mcpA* gene can confer upon *P. putida* F1 the ability to positively respond to TCE (4). Parales et al. reported that positive chemotactic responses to TCE by *P. putida* F1 are induced by toluene and that *P. putida* F1 cells grown in the absence of toluene fail to respond to TCE (10). We confirmed that *P. putida* F1 did not positively respond to 1 mM TCE when it was grown in the minimal medium in the absence of toluene (Fig. 2). Because this strain is resistant to carbenicillin, we exchanged the carbenicillin resistance marker in plasmid pHEK01 with a kanamycin resistance marker to construct plasmid pHEK02 and then introduced it into *P. putida* F1. The resulting *P. putida* F1 (pHEK02) strain was attracted by 1 mM TCE even when grown in the absence of toluene (Fig. 2). These results confirm that McpA is the MCP for positive chemotaxis to TCE. To our knowledge, this is the first report of a chemosensory protein for positive chemotaxis to TCE.

We then investigated the specificity of McpA. Since *P. putida* F1 cells grown in the absence of toluene did not respond to chloroethylenes, including tetrachloroethylene (PCE), 1,1-dichloroethylene (1,1-DCE), 1,2-*cis*-dichloroethylene (1,2-*cis*-DCE), and 1,2-*trans*-dichloroethylene (1,2-*trans*-DCE), we examined *P. putida* F1(pHEK02) for responses to these compounds to investigate the specificity of McpA. *P. putida* F1(pHEK02) cells grown in the absence of toluene accumulated around agarose plugs containing these chloroethylenes but not around the control agarose plug (Fig. 2). Thus, it was demonstrated that McpA functions as an MCP for positive chemotaxis to TCE, PCE, and DCE isomers.

Ferrández et al. were the first to characterize *mcpA* (3). They found that the *mcpA* mutant strain behaved like the wild type in minimal medium soft agar plates containing the organic chemoattractants but that it showed defective chemotaxis in low-magnesium medium. Schuster et al. demonstrated that expression of the *mcpA* gene is controlled by the alternative sigma factor RpoS (12). The open reading frame of *mcpA* is predicted to encode a 390-residue protein with a molecular mass of 43 kDa. Residues 192 to 235 of McpA are 70% identical to the 44-amino-acid highly conserved domain of the *Escherichia coli* chemotaxis transducer Tsr (1). MCPs from phylogenetically diverse bacteria have been shown to possess this highly conserved domain (21), which is important for the interaction of MCPs with CheW and CheA (8). Typical MCPs possess two hydrophobic membrane-spanning regions in their N-terminal domains; however, like the aerotaxis transducer Aer (6), McpA is predicted to possess only one hydrophobic sequence (residues 5 to 57). Although the N-terminal domain of McpA is identical to that of the *P. aeruginosa* PA14 McpA homologue (Paer03004584), it has no significant similarity to

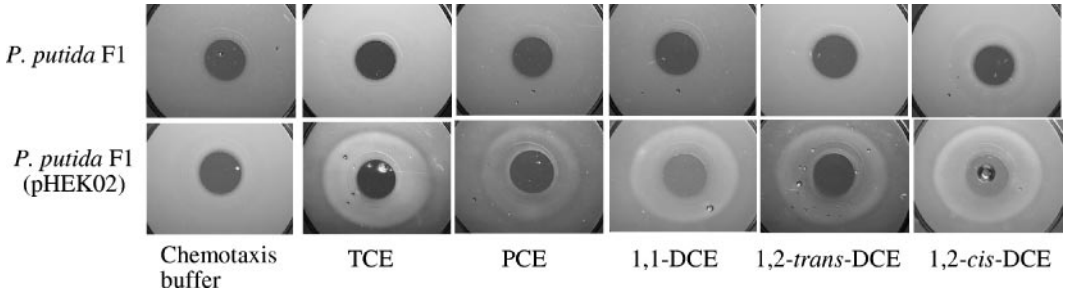

FIG. 2. Chemotactic responses of *P. putida* F1 and *P. putida* F1 harboring pHEK02 (carrying *mcpA*) to chloroethylenes in agarose plug assays. Agarose plugs contained 1 mM TCE, 0.1 mM PCE, 2 mM 1,1-DCE, 2 mM 1,2-*trans*-DCE, or 2 mM 1,2-*cis*-DCE. *P. putida* cells grown at 28°C in T_2 minimal medium were used for these assays.

any other known proteins. Because genetic analysis demonstrated the involvement of *mcpA* in positive TCE chemotaxis, we have renamed *mcpA cttP* (for "chemotactic transducer for TCE [positive chemotaxis]").

REFERENCES

1. **Boyd, A. W., K. Kendall, and M. I. Simon.** 1983. Structure of the serine chemoreceptor in *Escherichia coli.* Nature **301:**623–626.
2. **Croft, L., S. A. Beaston, C. B. Whiychurch, B. Huang, R. L. Blakeley, and J. Mattick.** 2000. An interactive web-based *Pseudomonas aeruginosa* genome database: discovery of new genes, pathways and structures. Microbiology **146:**2351–2364.
3. **Ferrández, A., A. C. Hawkins, D. T. Summerfield, and C. S. Harwood.** 2002. Cluster II *che* genes from *Pseudomonas aeruginosa* are required for an optimal chemotaxis response. J. Bacteriol. **184:**4374–4383.
4. **Finnette, B. A., V. Subramanian, and D. T. Gibson.** 1984. Isolation and characterization of *Pseudomonas putida* PpF1 mutants defective in the toluene dioxygenase enzyme system. J. Bacteriol. **160:**1003–1009.
5. **Holloway, B. W., V. Krishnapillai, and A. F. Morgan.** 1979. Chromosomal genetics of *Pseudomonas.* Microbiol. Rev. **43:**73–102.
6. **Hong, C. S., M. Shitashiro, A. Kuroda, T. Ikeda, N. Takiguchi, H. Ohtake, and J. Kato.** 2004. Chemotaxis proteins and transducers for aerotaxis in *Pseudomonas aeruginosa.* FEMS Microbiol. Lett. **231:**247–252.
7. **Kuroda, A., T. Kumano, K. Taguchi, T. Nikata, J. Kato, and H. Ohtake.** 1995. Molecular cloning and characterization of a chemotactic transducer gene in *Pseudomonas aeruginosa.* J. Bacteriol. **177:**7019–7025.
8. **Liu, J., and J. S. Parkinson.** 1991. Genetic evidence for interaction between the CheW and Tsr proteins during chemoreceptor signaling by *Escherichia coli.* J. Bacteriol. **173:**4941–4951.
9. **Nikata, T., K. Sumida, J. Kato, and H. Ohtake.** 1992. Rapid method for analyzing bacterial behavioral responses to chemical stimuli. Appl. Environ. Microbiol. **58:**2250–2254.
10. **Parales, R. E., J. L. Ditty, and C. S. Harwood.** 2000. Toluene-degrading bacteria are chemotactic toward the environmental pollutants benzene, toluene, and trichloroethylene. Appl. Environ. Microbiol. **66:**4098–4104.
11. **Parales, R. E., and C. S. Harwood.** 2002. Bacterial chemotaxis to pollutants and plant-derived aromatic molecules. Curr. Opin. Microbiol. **5:**266–273.
12. **Schuster, M., A. C. Hawkins, C. S. Harwood, and E. P. Greenberg.** 2004. The *Pseudomonas aeruginosa* RpoS regulon and its relationship to quorum sensing. Mol. Microbiol. **51:**973–985.
13. **Schweizer, H. P.** 1991. *Escherichia-Pseudomonas* shuttle vectors derived from pUC18/19. Gene **97:**109–121.
14. **Scott, C. S., and V. J. Cogliano.** 2000. Trichloroethylene health risks—state of the science. Environ. Health Perspect. **108**(Suppl. 2)**:**159–160.
15. **Shitashiro, M., T. Fukumura, J. Kato, A. Kuroda, T. Ikeda, N. Takiguchi, and H. Ohtake.** 2003. Evaluation of bacterial aerotaxis for its potential use in detecting toxicity of chemicals to microorganisms. J. Biotechnol. **101:**11–18.
16. **Shitashiro, M., H. Tanaka, C. S. Hong, A. Kuroda, N. Takiguchi, H. Ohtake, and J. Kato.** 2005. Identification of chemosensory proteins for trichloroethylene in *Pseudomonas aeruginosa.* J. Biosci. Bioeng. **99:**396–402.
17. **Taguchi, K., H. Fukutomi, A. Kuroda, J. Kato, and H. Ohtake.** 1997. Genetic identification of chemotactic transducers for amino acids in *Pseudomonas aeruginosa.* Microbiology **143:**3223–3229.
18. **Varder, G., P. Barbieri, and T. K. Wood.** 2005. Chemotaxis of *Pseudomonas stutzeri* OX1 and *Burkholderia cepacia* G4 toward chlorinated ethenes. Appl. Microbiol. Biotechnol. **66:**696–701.
19. **Wu, H., J. Kato, A. Kuroda, T. Ikeda, N. Takiguchi, and H. Ohtake.** 2000. Identification of two chemotactic transducers for inorganic phosphate in *Pseudomonas aeruginosa.* J. Bacteriol. **182:**3400–3404.
20. **Yu, H. S., and M. Alam.** 1997. An agarose-in-plug bridge method to study chemotaxis in the Archaeon *Halobacterium salinarum.* FEMS Microbiol. Lett. **156:**265–269.
21. **Zhulin, I. B.** 2001. The superfamily of chemotaxis transducers: from physiology to genomics and back. Adv. Microb. Physiol. **45:**157–198.