JOURNAL OF BACTERIOLOGY, Feb. 1995, p. 948–952
0021-9193/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Isolation and Characterization of Chemotaxis Mutants and Genes of *Pseudomonas aeruginosa*

AGUS MASDUKI, JIN NAKAMURA, TOMOMI OHGA, RYUICHI UMEZAKI, JUNICHI KATO, AND HISAO OHTAKE*

Department of Fermentation Technology, Hiroshima University, Higashi-Hiroshima, Hiroshima 724, Japan

Received 29 September 1994/Accepted 1 December 1994

Two chemotaxis-defective mutants of *Pseudomonas aeruginosa*, designated PC1 and PC2, were selected by the swarm plate method after *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine mutagenesis. These mutants were fully motile but incapable of swarming, suggesting that they had a defect in the intracellular signalling pathway. Computer-assisted capillary assays confirmed that they failed to show behavioral responses to chemical stimuli, including peptone, methyl thiocyanate, and phosphate. Two chemotaxis genes were cloned by phenotypic complementation of PC1 and PC2. From nucleotide sequence analysis, one gene was found to encode a putative polypeptide that was homologous to the enteric CheZ protein, while the other gene was *cheY*, which had been previously reported (M. N. Starnbach and S. Lory, Mol. Microbiol. 6:459–469, 1992). Deletion and complementation analysis showed that PC1 was a *cheY* mutant, whereas PC2 had a double mutation in the *cheY* and *cheZ* genes. A chromosomal *cheZ* mutant, constructed by inserting a kanamycin resistance gene cassette into the wild-type gene, changed its swimming direction much more frequently than did wild-type strain PAO1. In contrast, *cheY* mutants were found to rarely reverse their swimming directions.

Chemotaxis is the movement of an organism toward chemical attractants and away from chemical repellents (1). *Pseudomonas aeruginosa* is attracted to various amino acids (6) and is less strongly responsive to sugars and organic acids (15, 16). The taxis toward several amino acids is subject to control by nitrogen availability in a manner similar to the control of various enzymes of nitrogen metabolism (6) and is mediated by methylation and demethylation of methyl-accepting proteins analogous to those of the enteric bacteria (5). The strengths of the chemotactic responses to glucose and to citrate are also dependent on prior growth of the bacterial cells on those carbon sources (16). The glucose-binding protein has been identified as the glucose chemoreceptor in this organism (28). However, virtually nothing is known about the other chemoreceptors of *P. aeruginosa*. Our previous work demonstrated that *P. aeruginosa* is attracted to P_i (10). This chemotactic response is induced by P_i limitation. The P_i-starved cells are also attracted to arsenate (11). Since P_i competitively inhibits the response to arsenate, both P_i and arsenate are likely to be detected by the same chemoreceptor. Genetic evidence showed that P_i taxis in *P. aeruginosa* is not regulated by the *phoB* and *phoR* gene products but requires the *phoU* gene (12). *P. aeruginosa* is also repelled by thiocyanic and isothiocyanic esters, including allyl isothiocyanate, ethyl thiocyanate, methyl isothiocyanate, and methyl thiocyanate (19).

The molecular mechanisms that underlie bacterial chemotaxis have been studied intensively with the enteric bacteria *Escherichia coli* (20) and *Salmonella typhimurium* (30) and to a lesser extent with the gram-positive bacterium *Bacillus subtilis* (4). However, little is known about the chemotaxis genes in the bacterial species belonging to the genus *Pseudomonas*, and only an open reading frame (ORF) encoding the *P. aeruginosa cheY*-like gene, immediately downstream of the *fliA* gene, has been previously found (27). In this work, we isolated and characterized *P. aeruginosa* mutants defective in chemotaxis. We then cloned the *P. aeruginosa cheY* and *cheZ* genes by phenotypic complementation of these mutants.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are described in Table 1. *E. coli* strains, which were used for plasmid construction and DNA manipulation, were grown at 37°C with shaking in 2× YT medium (23) supplemented with appropriate antibiotics. This medium was also used for the preparation of *P. aeruginosa* cells for electroporation. Whenever necessary, 2× YT medium was supplemented with 5 mM IPTG (isopropyl-β-D-thiogalactopyranoside).

Chemotaxis assays. (i) Swarm plate method. The swarm plate method was an adaptation of that of Armstrong et al. (3). Tryptone swarm plates contained 1% tryptone (Difco), 0.5% NaCl, and 0.3% agar (Difco). Swarm plates were inoculated with a single colony, incubated at 37°C, and then examined for ring formation at intervals of over 24 h. Obvious rings were usually formed within a few hours.

(ii) Computer-assisted capillary method. Digital image processing was used to count the number of bacteria accumulating toward the mouth of a capillary containing a known concentration of an attractant plus 1% agarose (18). The strength of chemotactic response was determined by the number of bacteria per videotape frame. The chemotaxis buffer used was 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N′*-ethanesulfonic acid) buffer (pH 7.0).

Motility traces. Bacteria were viewed with an inverted phase-contrast microscope (IMT-2-HP; Olympus Co.) at a magnification of ×300. The video image was recorded continuously by a video camera (FCD-725; Ikegami Co.) and a video cassette recorder (BR9000; Hitachi Co.). Run times were measured as previously described (14). Measurements were made on the monitor screen by stretching plastic wrap flat against the screen surface and following a cell with a permanent marking pen. This process was timed, and the same path could be rerun to check the timing. This gave values for the run time and the frequency of direction reversal.

Isolation of chemotaxis mutants. Bacteria mutagenized with *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (9) were grown in 2× YT medium at 37°C for 5 h with shaking, harvested by centrifugation, and washed with 10 mM phosphate buffer (pH 7). Washed cells were suspended in molten tryptone swarm agar after sequential dilution, and the mixture was solidified by being dispensed into dishes. After 24 h of incubation at 37°C, bacteria which formed dense and small colonies were selected for further study.

DNA manipulation and sequencing. Standard procedures were used for plasmid DNA preparations, restriction enzyme digestions, ligations, transformations, and agarose gel electrophoresis (23). *P. aeruginosa* chromosomal DNA was prepared as described by Goldberg and Ohman (8). The nucleotide sequences of both strands were determined by the dideoxynucleotide sequencing method with single-stranded DNA (24, 31). A DNA sequence similarity search was done with the program FASTA (22) with GenBank.

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Relevant characteristics	Reference or source
E. coli		
MV1184	*ara* Δ(*lac-proAB*) *rpsL thi* (φ80 *lacZ*ΔM15) Δ(*srl-recA*)*306*::Tn*10* Tet^r F′[*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	31
SRB	*sbcC recJ umuC*::Tn*5 uvrC thi-1 endA1 supE44 lac gyrA96 relA1 mcrA* Δ(*mrr hsdRMS mcrBC*) F′[*proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	Stratagene
P. aeruginosa		
PAO1	Prototroph, FP$^-$	
PC1	PAO1 derivative, nonchemotactic mutant	
PC2	PAO1 derivative, nonchemotactic mutant	
CHEZ1	PAO1 derivative, ORF2::Kan^r	
Plasmids		
pUC119	Cloning vector; Ap^r *lacPOZ′*	31
pUC4K	pUC4 containing 1.3-kb Kan^r cartridge; Ap^r Kan^r	Pharmacia
pBluescriptII KS$^+$	Cloning vector; Ap^r *lacPOZ′*	Stratagene
pCP19	Broad-host-range cosmid; Tet^r IncP	9
pMMB66EH	Broad-host-range vector; Cb^r P_{tac} *lacI*^{q} IncQ	7
pME1	pUC12 containing 8.3-kb *Pst*I fragment from *S. typhimurium*; Ap^r *cheA cheW tar cheR cheB cheY cheZ flaM*	29
pPT07	pCP19 harboring 20- and 7-kb *Hin*dIII fragments from *P. aeruginosa* PAO1; Tet^r	This study
pPT07.1	pCP19 harboring 15-kb *Bam*HI-*Hin*dIII fragment from pPT07; Tet^r	This study
pPT07.2	pCP19 harboring 3.6-kb *Bam*HI-*Xho*I fragment from pPT07; Tet^r	This study
pPT07.3	pCP19 harboring 2.6-kb *Bam*HI-*Eco*RI fragment from pPT07; Tet^r	This study
pPT07.4	Deleted version of pPT07.3; the 2.6-kb insertion fragment of pPT07.3 was deleted from the *Eco*RI site by BAL 31 nuclease (deletion size was 0.1 kb); Tet^r	This study
pPT07.5	pCP19 harboring 1.7-kb *Cla*I-*Hin*dIII fragment from pME1; Tet^r *cheY*	This study
pPT07.6	pMMB66EH harboring 2-kb *Xho*I fragment from pPT07.2; Cb^r P_{tax}-*CheY*-ORF2 *lacI*^{q}	This study
pPT07.7	pMMB66EH harboring 1-kb *Eco*RI-*Xho*I fragment from pPT07.6; Cb^r P_{tac}-ORF1 *lacI*^{q}	This study
pPT07.8	pMMB66EH harboring 0.9-kb *Sac*I-*Xho*I fragment from pPT07.6; Cb^r *lacI*^{q}	This study
pPT07.9	pMMB66Eh harboring 1.7-kb *Ssp*I-*Pst*I fragment from pME1; Cb^r P_{tac}-*CheZ lacI*^{q}	This study
pPT07.10	pBluescriptII KS$^+$ harboring 4.9-kb *Bam*HI-*Sal*I fragment from pPT07; Ap^r	This study
pPT07.11	pPT07.8 derivative containing ORF1 disrupted by insertion of Kan^r cartridge; pPT07.8 was partially digested with *Hin*cII and ligated with 1.3-kb Kan^r cartridge from pUC4K; Ap^r ORF1::Kan^r	This study

Electroporation. *P. aeruginosa* was transformed by electroporation. Bacterial cells, grown overnight in 2× YT medium, were inoculated into fresh 2× YT medium (a 1% inoculum), and after 2 h of incubation, cells were harvested by centrifugation (10,000 × *g*, 5 min, 4°C). Pelleted cells were resuspended in ice-cold HS buffer (7 mM HEPES plus 252 mM sucrose, pH 7.0), washed three times with the same buffer, and resuspended in a 0.1 original volume of HS buffer. Electroporation was performed with an Electro Cell Manipulator 600 (BTX Inc., San Diego, Calif.) at a 50-μF capacitance and 1.3 kV. After electroporation, cells were allowed to grow in 2× YT medium for 2 h and spread on appropriate solid media to obtain transformants.

Disruption of the chromosomal *cheZ* gene. A 4.9-kb *Bam*HI-*Sal*I fragment of plasmid pPT07 was inserted into pBluescriptII KS$^+$ to construct pPT07.10. Plasmid pPT07.10 was then digested with *Hin*cII and ligated with a *Hin*cII-flanked kanamycin resistance (Kan^r) gene cassette from pUC4K. The resulting plasmid, designated pPT07.11, was introduced into *P. aeruginosa* PAO1 by electroporation, and Kan^r transformants were selected on 2× YT plates with kanamycin. The disruption of the chromosomal *cheZ* gene was confirmed by Southern hybridization (26).

Nucleotide sequence accession number. The nucleotide sequence of the *cheZ* gene has been deposited in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases under accession number D37810.

RESULTS AND DISCUSSION

Isolation of chemotaxis mutants. Thirty-two putative mutants showing impaired swarming ability were picked, subcultured, and retested. The putative behavioral mutants were then subjected to further analysis by phase-contrast microscopy. Microscopic analysis revealed that two of the nonswarming mutants, designated PC1 and PC2, were motile but rarely reversed swimming directions (Table 2). Both mutants grew as well as the parent strain PAO1, confirming that the impaired swarming ability was not due to a metabolic defect. Computer-assisted capillary assays showed that these mutants were not attracted to peptone (Fig. 1). Neither methyl thiocyanate nor P_i elicited a tactic response from PC1 or PC2 (data not shown). These results suggested that PC1 and PC2 had a defect in the intracellular signalling pathway.

Complementation of chemotaxis mutations. A genomic library from *P. aeruginosa* PAO1 was prepared in the broad-host-range cosmid vector pCP19 (9), and PC1 was transformed with this genomic library. Transformants were selected on swarm plates with tetracycline. One transformant, which formed large swarms, was selected for further study. A recombinant cosmid, designated pPT07 (Fig. 2), was isolated from this transformant. Plasmid pPT07 was then reintroduced into PC1, and it was confirmed that the transformants complemented the mutation of PC1. The genomic origin of the pPT07 insert was confirmed by Southern blot analysis of *P. aeruginosa*

TABLE 2. Frequency of reversal of swimming direction[a]

Strain[b]	Changes of direction/s[c]
PAO1	0.9 ± 0.4
CHEZ1	2.7 ± 0.4
CHEZ1(pPT07.7)	1.4 ± 0.6
CHEZ1(pPT07.8)	2.3 ± 0.5
CHEZ1(pPT07.9)	0.8 ± 0.6
PC1	<0.1
PC1(pPT07)	0.8 ± 0.1
PC2	<0.1

[a] The motile behavior of *P. aeruginosa* cells suspended in HEPES buffer (pH 7) was observed with an inverted phase-contrast microscope.

[b] Cells were grown in 2× YT medium. For CHEZ1 transformants, 2× YT medium was supplemented with carbenicillin and IPTG.

[c] The means ± standard deviations of values obtained fro 25 individual swimming cells are shown. At least 5 s of behavior was observed in each analysis.

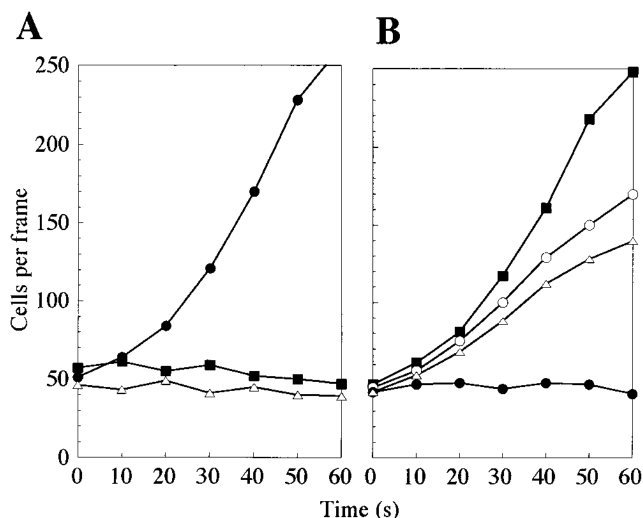

FIG. 1. (A) Chemotactic responses of *P. aeruginosa* wild-type PAO1 (circles) and the chemotactic mutants PC1 (triangles) and PC2 (squares) to peptone. Digital image processing was used to count the number of bacteria accumulating toward the mouth of a capillary containing 0.1% peptone plus 1% agarose. One videotape frame was analyzed at each time point, and the chemotactic response is presented as the number of bacteria per videotape frame as previously described (18). (B) Chemotactic responses of *P. aeruginosa* PC1(pPT07) (squares), PC1(pPT07.3) (open circles), PC1(pPT07.4) (solid circles), and PC1(pPT07.5) (triangles) to peptone.

PAO1 chromosomal DNA (data not shown). Computer-assisted capillary assays revealed that PC1(pPT07) was attracted to peptone (Fig. 1).

Restriction endonuclease analysis revealed that pPT07 contained two *Hin*dIII fragments of approximately 7 and 20 kb. The two *Hin*dIII fragments were subcloned into pCP19. The recombinant plasmid pPT07.1, which carried the 20-kb *Hin*dIII fragment, complemented the mutation of PC1 (Fig. 2). The 20-kb insert of pPT07.1 was then digested with various restriction enzymes, and the fragments were subcloned into pCP19 (Table 1 and Fig. 2). The 2.0-kb *Xho*I fragment in pPT07.6 complemented the mutations of both PC1 and PC2. Plasmid pPT07.3 restored the ability of PC1 to respond to peptone (Fig. 1) but failed to complement the mutation of PC2. Neither mutation was complemented by pPT07.7.

DNA sequence analysis. Nucleotide sequence analysis of the pPT07.6 insert revealed that the 2.0-kb *Xho*I fragment contained two potential reading frames (ORF1 and ORF2) (Fig. 2). A computer-assisted homology search showed that ORF1 encoded the *P. aeruginosa* homolog of the enterobacterial *cheY* gene, which had been previously reported (27). ORF2 contained a putative GTG start codon at base positions 80 to 82 (Fig. 3A). A 128-bp intercistronic DNA region was located between the end of the *cheY*-like gene and the proposed initiator codon for ORF2. ORF2 was preceded by a putative ribosome-binding site (Fig. 3A, underline). Analysis of the amino acid sequence revealed that the putative polypeptide encoded by ORF2 shared 39% identical amino acid residues with the product of the *E. coli cheZ* gene (Fig. 3B). According to the description by Mutoh and Simon (17), the *E. coli cheZ* gene encodes a polypeptide corresponding to 214 amino acid residues with a molecular mass of 24 kDa. The *P. aeruginosa cheZ*-like gene encoded a polypeptide comprising 227 amino acid residues with a molecular mass of 25 kDa. Inspection of the 3′ noncoding sequence of the 2.0-kb *Xho*I fragment shows no putative transcriptional termination signal, suggesting the existence of other ORFs downstream of the *cheZ*-like gene.

Characterization of *cheY* and *cheZ* genes. Although the *P. aeruginosa cheY*-like gene has been previously found immediately downstream of the *fliA* gene, which encodes a sigma factor required for flagellin synthesis (27), there is no direct evidence supporting the involvement of this gene in chemotactic signal transduction in *P. aeruginosa*. Deletion derivative plasmids of pPT07.3, which contained the entire *cheY*-like gene but not the *cheZ*-like gene, were constructed by using BAL 31. Plasmid pPT07.4, which carried a 40-bp deletion from the 3′ end of the *cheY*-like gene (Fig. 3A), could not restore the ability of PC1 to respond to peptone (Fig. 1). To test whether the enterobacterial *cheY* gene can complement the mutation of PC1, the *S. typhimurium cheY* gene was excised from pME1 (29) and inserted in pCP19 to make pPT07.5. When plasmid pPT07.5 was introduced into PC1, this plasmid partially complemented the mutation of PC1 (Fig. 1). This evidence confirmed that the ORF1 gene encodes the *P. aeruginosa cheY* gene and that the

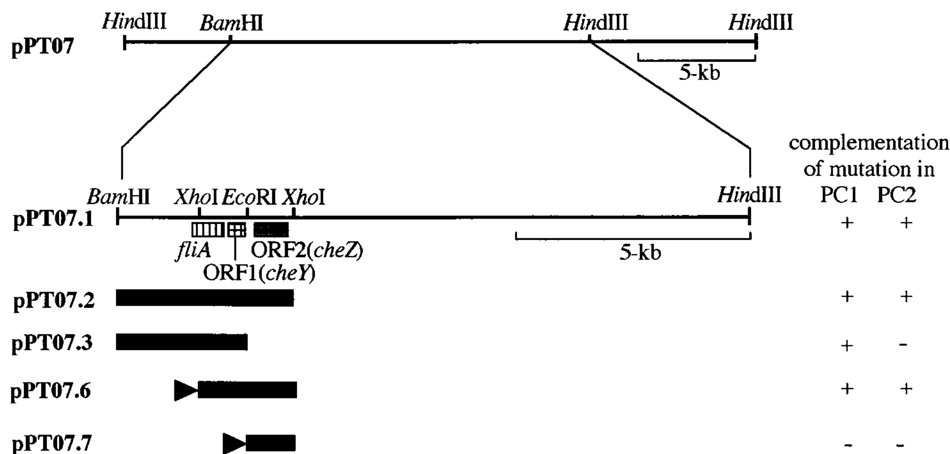

FIG. 2. Restriction map of plasmid pPT07, containing two *Hin*dIII fragments of 7 and 20 kb from the *P. aeruginosa* chromosomal DNA, and the subclones of plasmid pPT07. The locations of *fliA*, ORF1 (*cheY*), and ORF2 (*cheZ*) are indicated below the restriction map of pPT07.1. Solid bars indicate *P. aeruginosa* chromosomal DNA fragments subcloned into vector plasmids. Arrowheads show the sites and directions of the *tac* promoter. The abilities of recombinant plasmids to complement the mutations of PC1 and PC2 are indicated on the right.

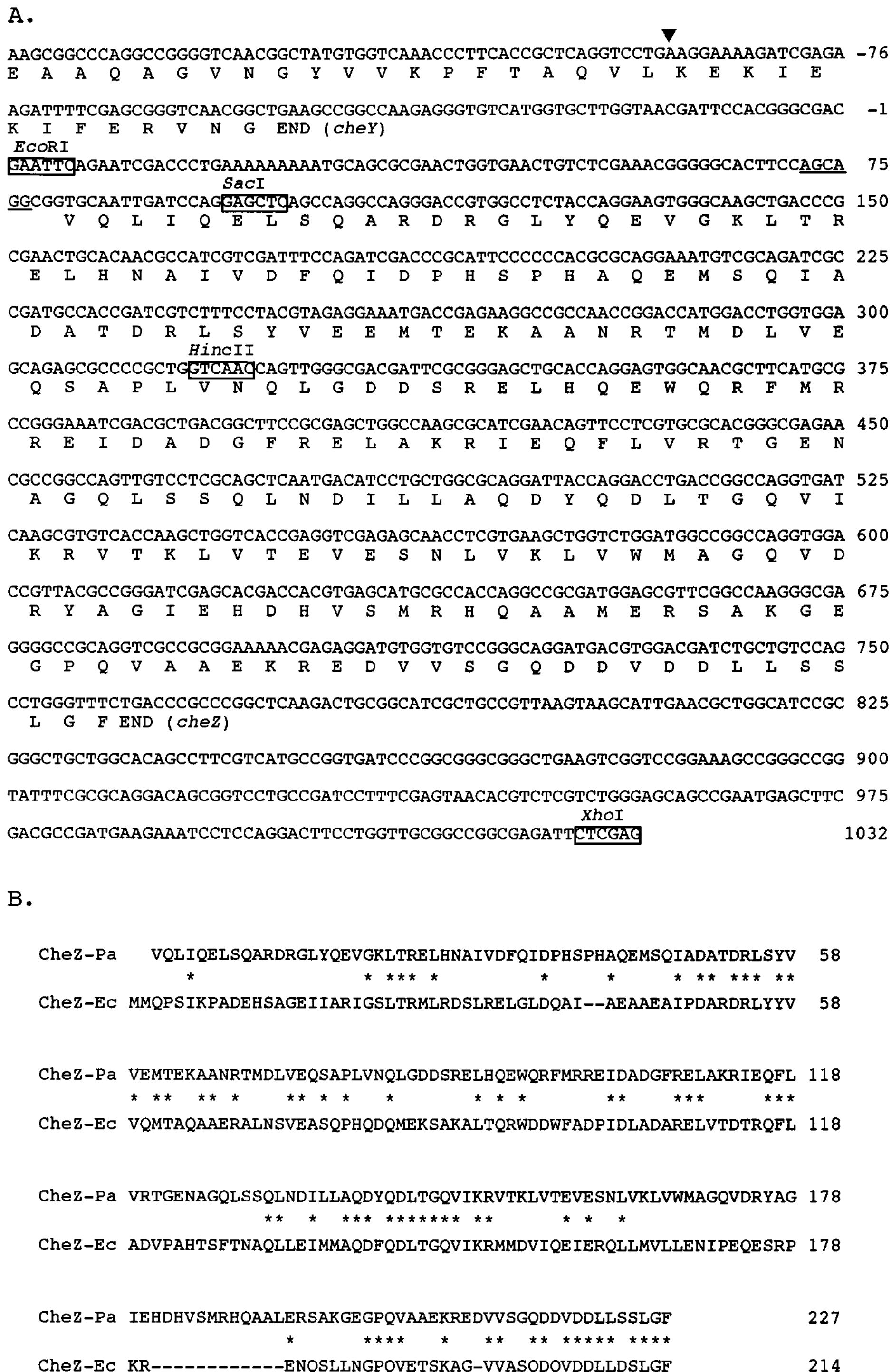

FIG. 3. (A) Nucleotide sequence of the *P. aeruginosa cheZ* coding region and flanking sequences. Numbering starts at the *Eco*RI site. The start codon GTG is located at positions 80 to 82, and the stop codon is at positions 761 to 763. A putative ribosome-binding site is underlined. Amino acids deduced from the nucleotide sequence are specified by standard one-letter abbreviations. Relevant restriction sites are located above the sequence. An inverted triangle shows the start site of a 40-bp sequence which was removed from the 3′ end of *cheY* with BAL 31 to make pPT07.4. (B) Amino acid sequence comparison of the *E. coli* (Ec) and *P. aeruginosa* (Pa) *cheZ* genes. The alignment was performed with the FASTA and Best-Fit programs (22). Identical amino acids (asterisks) and gaps (dashes) introduced into the sequence to maximize the alignment are indicated.

mutation of the *cheY* gene renders *P. aeruginosa* cells nonchemotactic. The mutation of PC2 was complemented by the 2.0-kb *Xho*I insert of pPT07.6 but not by the 2.6-kb *Bam*HI-*Eco*RI insert of pPT07.3 (Fig. 2). The 1-kb *Eco*RI-*Xho*I fragment, containing only the *cheZ*-like gene, was excised from pPT07.6 and inserted downstream of the *tac* promoter of pMMB66EH to make pPT07.7. The 1-kb *Eco*RI-*Xho*I insert of pPT07.7 failed to complement the mutation of PC2, indicat-

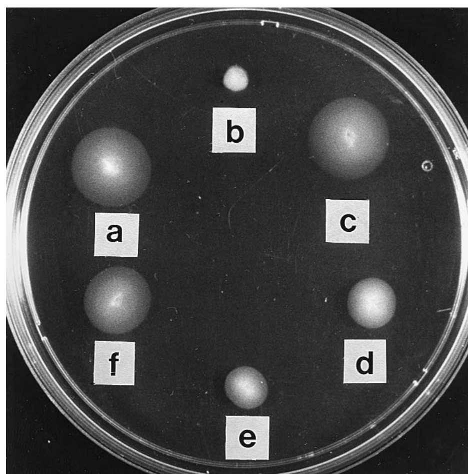

FIG. 4. Swarm plate phenotypes of *P. aeruginosa* strains. (a) PAO1; (b) PC1; (c) CHEZ1(pPT07.7); (d) CHEZ1(pPT07.8); (e) CHEZ1; (f) CHEZ1(pPT07.9).

ing that PC2 has a double mutation of the *cheY* and *cheZ* genes.

To characterize the *cheZ*-like gene, a mutation of the chromosomal gene was constructed by inserting a Kan^r gene cassette into the wild-type gene (see Materials and Methods). The results of the Southern analysis confirmed that the *cheZ*-like gene was properly interrupted at a *Hin*cII site (positions 236 to 242 in Fig. 3A) by the Kan^r gene cassette (data not shown). The chromosomal mutant, designated CHEZ1, formed smaller swarms than did the parental controls (Fig. 4). Microscopic analysis revealed that CHEZ1 cells were fully motile but changed swimming direction much more frequently than did the parental strain PAO1 (Table 2). The 1.7-kb *Ssp*I-*Pst*I fragment, which carried the *S. typhimurium cheZ* gene, was excised from pME1 (29) and inserted in pMMB66EH (7) to make pPT07.9. CHEZ1(pPT07.9) formed larger swarms than did CHEZ1 (Fig. 4), indicating that the *S. typhimurium cheZ* gene could partially complement the mutation of CHEZ1. There was a *Sac*I endonuclease recognition site 13 to 18 bp downstream of the putative GTG start codon of ORF2 (Fig. 3A). To confirm the essentiality of the GTG start codon, the 0.9-kb *Sac*I-*Xho*I fragment was excised from pPT07.6 and inserted in pMMB66EH to make pPT07.8. This recombinant plasmid carried a 12-bp deletion from the 5′ end of ORF2 (Fig. 3A). CHEZ1 (pPT07.8) formed smaller swarms than did PAO1, while pPT07.7, which carried the entire ORF2, restored the ability of CHEZ1 to form swarm rings (Fig. 4). These findings confirmed that the ORF2 gene encodes the *P. aeruginosa cheZ* gene.

In *E. coli*, phosphorylation of CheY protein is the signal required to switch flagellar rotation from counterclockwise to clockwise (13, 25). This causes a change from a smooth-swimming phenotype to a tumbling phenotype. *E. coli* with a null mutation of *cheY* has extremely smooth swimming behavior, whereas *E. coli cheZ* mutants tumble excessively (21). Similar behaviors were also observed with *P. aeruginosa cheY* and *cheZ* mutants (Table 2). *P. aeruginosa* possesses a single polar flagellum, and reversals in the direction of rotation of the polar flagellum result in changes in swimming direction (2). On the basis of the observations in this study, it is likely that *P. aeruginosa* CheY and CheZ are involved in changing the direction of flagellar rotation.

ACKNOWLEDGMENTS

This work was supported in part by a research grant from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. **Adler, J.** 1966. Chemotaxis in bacteria. Science **153:**708–716.
2. **Armitage, J. P., and M. C. Evans.** 1983. The motile and tactic behaviour of *Pseudomonas aeruginosa* in anaerobic environment. FEBS Lett. **156:**113–118.
3. **Armstrong, J. B., J. Adler, and M. M. Dahl.** 1973. Nonchemotactic mutants of *Escherichia coli*. J. Bacteriol. **93:**390–398.
4. **Bischoff, D. S., and G. W. Ordal.** 1992. *Bacillus subtilis* chemotaxis: a deviation from *Escherichia coli* paradigm. Mol. Microbiol. **6:**23–28.
5. **Craven, R. C., and T. Montie.** 1983. Chemotaxis of *Pseudomonas aeruginosa*: involvement of methylation. J. Bacteriol. **154:**780–786.
6. **Craven, R. C., and T. Montie.** 1985. Regulation of *Pseudomonas aeruginosa* chemotaxis by the nitrogen source. J. Bacteriol. **164:**544–549.
7. **Furste, J. P., W. Pansegrau, R. Frank, H. Blocker, P. Scholz, M. Bagdasarian, and E. Lanka.** 1986. Molecular cloning of the plasmid RP4 primase region in a multi-host-range *tacP* expression vector. Gene **48:**119–131.
8. **Goldberg, J. B., and D. E. Ohman.** 1984. Cloning and expression in *Pseudomonas aeruginosa* of a gene involved in the production of alginate. J. Bacteriol. **158:**1115–1121.
9. **Goldberg, J. B., and D. E. Ohman.** 1987. Construction and characterization of *Pseudomonas aeruginosa algB* mutants: role of AlgB in high-level production of alginate. J. Bacteriol. **169:**1593–1602.
10. **Kato, J., A. Ito, T. Nikata, and H. Ohtake.** 1992. Phosphate taxis in *Pseudomonas aeruginosa*. J. Bacteriol. **174:**5149–5151.
11. **Kato, J., T. Nikata, A. Masduki, K. Taguchi, Y. Sakai, and H. Ohtake.** 1994. Phosphate taxis and its regulation in *Pseudomonas aeruginosa*, p. 315–317. *In* A. Torriani-Gorini, E. Yagil, and S. Silver (ed.), Phosphate in microorganisms: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
12. **Kato, J., Y. Sakai, T. Nikata, and H. Ohtake.** 1994. Cloning and characterization of a *Pseudomonas aeruginosa* gene involved in the negative regulation of phosphate taxis. J. Bacteriol. **176:**5874–5877.
13. **Kuo, S. C., and D. E. Koshland, Jr.** 1987. Roles of *cheY* and *cheZ* gene products in controlling flagellar rotation in bacterial chemotaxis of *Escherichia coli*. J. Bacteriol. **169:**1307–1314.
14. **Mitchell, J. G., M. Martinez-Alonso, J. Lalucat, I. Esteve, and S. Brown.** 1991. Velocity changes, long runs, and reversals in the *Chromatium minus* swimming response. J. Bacteriol. **173:**997–1003.
15. **Moench, T. T., and W. A. Konetzka.** 1978. Chemotaxis in *Pseudomonas aeruginosa*. J. Bacteriol. **133:**427–429.
16. **Moulton, R. C., and T. C. Montie.** 1978. Chemotaxis by *Pseudomonas aeruginosa*. J. Bacteriol. **137:**274–280.
17. **Mutoh, N., and M. I. Simon.** 1986. Nucleotide sequence corresponding to five chemotaxis genes in *Escherichia coli*. J. Bacteriol. **165:**161–166.
18. **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.
19. **Ohga, T., A. Masduki, J. Kato, and H. Ohtake.** 1993. Chemotaxis away from thiocyanic and isothiocyanic esters in *Pseudomonas aeruginosa*. FEMS Microbiol. Lett. **113:**63–66.
20. **Parkinson, J. S.** 1978. Complementation analysis and deletion mapping of *Escherichia coli* mutants defective in chemotaxis. J. Bacteriol. **135:**45–53.
21. **Parkinson, J. S., S. R. Parker, P. B. Talbert, and S. E. Houts.** 1983. Interactions between chemotaxis genes and flagellar genes in *Escherichia coli*. J. Bacteriol. **155:**265–274.
22. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
23. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
25. **Segall, J. E., M. D. Manson, and H. C. Berg.** 1982. Signal processing times in bacterial chemotaxis. Nature (London) **296:**855–857.
26. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
27. **Starnbach, M. N., and S. Lory.** 1992. The *fliA* (*rpoF*) gene of *Pseudomonas aeruginosa* encodes an alternative sigma factor required for flagellin synthesis. Mol. Microbiol. **6:**459–469.
28. **Stinton, M. W., M. A. Cohen, and J. M. Merrick.** 1977. Purification and properties of the periplasmic glucose-binding protein of *Pseudomonas aeruginosa*. J. Bacteriol. **131:**672–681.
29. **Stock, A., J. Mottonen, T. Chen, and J. Stock.** 1987. Identification of a possible nucleotide binding site in CheW, a protein required for sensory transduction in bacterial chemotaxis. J. Biol. Chem. **262:**535–537.
30. **Stock, J. B., G. S. Lukat, and A. M. Stock.** 1991. Bacterial chemotaxis and the molecular logic of intracellular signal transduction networks. Annu. Rev. Biophys. Biophys. Chem. **20:**109–136.
31. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153:**3–11.