JOURNAL OF BACTERIOLOGY, Mar. 1989, p. 1631–1637
0021-9193/89/031631-07$02.00/0
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Vol. 171, No. 3

Nucleotide Sequence and Expression of *cheF*, an Essential Gene for Chemotaxis in *Bacillus subtilis*

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Received 23 September 1988/Accepted 9 December 1988

The *cheF* gene, which is involved in chemotaxis in *Bacillus subtilis*, has been cloned, expressed, and sequenced. This gene is contained in a 0.7-kilobase *Pst*I DNA fragment that was isolated from a λ Charon 4A *B. subtilis* chromosomal DNA library. This fragment was subcloned into the expression vector pSI-1 and shown to complement the *cheF* mutation both for chemotaxis and for methanol production in response to the addition of attractants. Plasmid-encoded DNA expression in *B. subtilis* maxicells indicated that a membrane-associated polypeptide of 20-kilodaltons was expressed from this 0.7-kilobase DNA. The nucleotide sequence of this DNA fragment was determined, and an open reading frame capable of encoding a putative 175-amino-acid protein (M_r 20,002) was identified. In an effort to understand the function of the *cheF* protein, the dosage of the *cheF* gene product was varied by altering the concentration of IPTG (isopropyl-β-D-thiogalactopyranoside) during growth. In the presence of high concentrations of IPTG, chemotaxis was inhibited and methanol production was impaired.

Bacterial chemotaxis is a primitive sensory mechanism by which bacteria swim toward higher concentrations of attractant and lower concentrations of repellent. Much of our knowledge about the pathway of information processing in bacterial chemotaxis is the result of work done on the gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium* (17, 21). However, recent experiments (26a) have suggested that the pathway of chemotactic signal processing might be quite different in the gram-positive *Bacillus subtilis*. Previous work suggests that methyl groups are transferred via one or more intermediate carriers from the methyl-accepting chemotaxis proteins (MCPs) to form methanol (26, 26a). Current evidence suggests that methanol evolves directly from MCPs in *E. coli* (21). In *B. subtilis*, methanol is generated in response to addition or removal of both attractants and repellents (26; M. Thoelke and G. Ordal, unpublished data). To explore the chemotactic mechanism in *B. subtilis*, a number of chemotactic mutants were isolated and characterized (19). More than 20 complementation groups were shown to be involved (20). All mutants are motile, and the corresponding genes except for *cheR* are located in the same region of the chromosome, between *thyA* and *pyrD*. Mutation in the *cheF* locus results in failure to respond to chemotactic stimuli (20). The region coding for the *cheF* protein was previously localized on a 7.7-kilobase segment isolated from a λ Charon 4A *B. subtilis* chromosomal DNA library (20). As part of a continuing investigation into the molecular mechanism involved in chemotaxis, the *cheF* gene was subcloned into an expression vector and its gene product was identified. This procedure allowed detection of the subcellular location of the *cheF* protein. The nucleotide sequence of the *cheF* gene was determined. To further analyze the gene product, the dosage of the *cheF* protein was varied in vivo, and the effects on chemotaxis and methanol production were quantitated.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. *E. coli* strains JM103 and DH5α were used for propagating plasmids. Strains JM103 and NM522 were the hosts for M13 phage vectors and pTZ phagemids, respectively. Complementation and expression studies were performed in isogenic strains in *B. subtilis* strain OI1925. The plasmid used for subcloning was pSI-1 (31). This shuttle plasmid contains a spac-I promoter and a *lacI* gene, which have been shown to function in *B. subtilis* (32). DNA fragments were subcloned into M13mp18 and M13mp19 phage vectors for nucleotide sequence analysis. Two phagemids, pTZ18U and pTZ19U, were also used as vectors for some DNA fragments in the sequencing study. Two phages (λ-14.9 and λ-11.7) harboring clustered *che* genes were isolated from a λ Charon 4A library (19). A 7.7-kilobase *Eco*RI DNA fragment from λ-11.7 was subcloned into pFH7 and shown to be capable of complementing *cheF* mutations (20). The *cheF* mutants defective in general chemotactic stimulation were identified by various chemotactic assays (20). This 7.7-kilobase *Eco*RI DNA fragment was then cloned into pUC18, and the restriction map of this fragment was determined (C. Ying and G. Ordal, unpublished results). This chimeric plasmid, designated pGO101, was subsequently used in our subcloning experiment. All strains and plasmids used are listed in Table 1.

Construction of *B. subtilis* strains. The chemotactic wild-type strain, OI1085, used in a previous study (29) was shown to be a poor host for plasmid DNA transformation. A strain which offered reasonable transformational frequency thus was crucial for subcloning and complementation. OI1878, a derivative of OI1085, was constructed (see Table 1 for construction procedure). This strain could be transformed with plasmid DNAs at a high frequency. The *cheF141* allele and subsequently the *recE4* allele were transferred into OI1878 (for constructions, see Table 1). This strain, OI1925, was used as the *cheF* mutant in the following studies.

Culture media and growth conditions. Unless otherwise stated, *E. coli* and *B. subtilis* were grown in L broth or on tryptone plates containing ampicillin or chloramphenicol (35

TABLE 1. Bacterial strains and plasmids used

Bacterial strain or plasmid	Genotype	Source or reference
Bacterial strain		
B. subtilis		
OI1085	*metC trpF7 hisH2* Che$^+$	29
MI112	*leuA8 argl5 thrA recE4* r_M^- m_M^-	25
OI363	*ilvC1 leu-1*	18
OI1878	*leu-1 trpF7*	This work[a]
M141	*metC trpF7 hisH2 cheF141*	19
IA302	*argC4 hisA1 recE4*	*Bacillus* Genetic Stock Center
OI1881	*trpF7 cheF141*	This work[b]
OI1925	*trpF7 cheF141 recE4*	This work[c]
OI1929	*trpF7 cheF141 recE4* pGO102	This work
OI1933	*trpF7 cheF141 recE4* pSI-1	This work
E. coli		
JM103	*supE thi* Δ(*lac-proAB*) (F′ *traD36 proAB lacI*^{q} *lacZ*ΔM15)	15
NM522	*hsd*Δ5 Δ(*lac-proAB*) *supE thi* (F′ *proAB lacI*^{q}*Z*ΔM15)	6
DH5α	F$^-$ *endA1 hsdR17* (r_K^- m_K^+) *supE44 thi1* λ$^-$ *recA1 gyrA96 relA1* φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169	9
Plasmid		
pGO101	Ap^r	G. W. Ordal, unpublished data
pSI-1	Cm^r	33
pGO102	Cm^r; a 700-bp *Pst*I fragment containing *cheF* was inserted into pSI-1 from pGO101	This work
pTZ18U	Ap^r	13
pTZ19U	Ap^r	13
pJH101	Ap^r Cm^r	4

[a] OI1085 competent cells were transformed to Met$^+$ with chromosomal DNA of MI112. One of these transformants has a high transformation frequency for pSI-1 due to cotransformation of the r_M^- or other genetic locus. This strain was subsequently made His$^+$ Leu$^-$ by using chromosomal DNA from OI363.

[b] The *cheF141* mutation was transferred from M141 chromosomal DNA into OI1878 competent cells, and the characteristic chemotactic phenotype of the *cheF141* mutation of this constructed strain, OI1881, was checked by chemotaxis assays.

[c] The allele of *recE4* was introduced into OI1881 competent cells from the chromosomal DNA of IA302 through gene cong with plasmid pSI-1. Colonies which were Cm^r were screened on plates containing 0.6 μl of ethyl methanesulfonate and 5 μg of chloramphenicol per ml. Ethyl methanesulfonate-sensistive colonies were identified and checked on swarm plates containing the same concentration of chloramphenicol. One of these colonies which showed the *cheF141* phenotype was chosen. Plasmid pSI-1 was then segregated from this strain. In this manner, OI1925, which contained the *cheF141* and *recE4* alleles, was constructed.

or 5 μg/ml, respectively) when needed for plasmid selection. The resuspension medium of Sterlini and Mandelstam (24) was used in maxicell experiments. Media used in transformation of *B. subtilis* were SPI and SPII (3). For capillary assays, bacteria were grown in minimal medium supplemented with 0.2% sorbitol and amino acids required for growth (20). Tryptone swarm plates and mannitol swarm plates were used as described previously (19). The protoplast, methyltransferase, and chemotaxis buffers were prepared according to the method of Ullah and Ordal (29).

Transformation. Transformation of *B. subtilis* with plasmid DNA was performed according to the method of Dubnau and Davidoff-Abelson (3). The method of Maniatis et al. (12) was used to transform *E. coli* with plasmid DNA.

Chemotactic assays of *B. subtilis* strains. Fresh transformants were picked from plates with toothpicks after overnight incubation and spotted onto tryptone swarm plates containing 5 μg of chloramphenicol per ml. Plates were incubated in a wet incubator for 5 to 12 h. The swarm phenotypes of each colony were observed. Capillary assays were performed as described previously (20). Capillary tubes filled with 3×10^{-5} M mannitol or 10^{-2} M aspartate were placed into a cell suspension. Reactions were continued for 45 min at 37°C. Bacteria accumulated inside the capillary tube were plated. After overnight incubation, the number of colonies on the plate was counted.

Changes of methanol evolution upon addition of attractants were detected in Conway diffusion cells (29). Bacteria were grown in L broth and harvested when the cell density was 180 Klett units. Cells were washed with chemotaxis buffer and suspended in protoplast buffer at a density of A_{600} = 1.0. Protoplasts of those cells were prepared by adding 1 mg of lysozyme per ml to the buffer. These protoplasts were labeled with L-[*methyl*-^{3}H]methionine for 5 min at 37°C and then mixed with 2.3×10^{-2} M aspartate in Conway diffusion cells. The reaction was stopped, and the amount of methanol released from cells was quantitated according to the method of Thoelke et al. (in press).

Plasmid-encoded DNA expression in *B. subtilis* maxicells. *B. subtilis* OI1925 was used as the host in this study. Cells were prepared and UV irradiated according to Shimotsu et al. (23) with few modifications. After the irradiation, cells were incubated at 37°C for 30 min followed by addition of 20 μg of D-cycloserine per ml, and incubation was continued for 16 to 24 h. Cells were then labeled with [^{35}S]methionine and lysed as described by Hirochika et al. (10). Labeled proteins were visualized by autoradiography.

To separate the membrane fraction from the cytoplasmic fraction of the plasmid-encoded proteins in maxicells, a modified method was used. Bacteria were grown, irradiated with UV light, and labeled with [^{35}S]methionine as described above. Protoplasts of these cells were made by adding 1 mg of lysozyme per ml to the cells suspended in 1 ml of protoplast buffer. Cells were incubated at 37°C for 45 min before they were harvested. Cell pellets were then lysed in 50 μl of water at 4°C. Membrane and cytoplasm fractions were separated by centrifugation in an airfuge at 100,000 × *g* for 45 min at 4°C. The membrane fraction precipitated in the pellet was suspended in 50 μl of 1 M NaCl prepared in methyltransferase buffer solution and centrifuged for another 45 min at 100,000 × *g* at 4°C. After this centrifugation, membrane-associated molecules which now remained in the supernatant were separated from membranes. Each fraction was suspended in sodium dodecyl sulfate solubilizer (62 mM Tris, 2% sodium dodecyl sulfate, 10% glycerol, 5% β-mercaptoethanol, pH 6.8), and cell debris was removed. Proteins in each fraction were fractionated on sodium dodecyl sulfate-polyacrylamide gels.

DNA preparation and analysis. The chromosomal DNA of *B. subtilis* was prepared by standard methods. Plasmid DNAs were isolated from *E. coli* or *B. subtilis* as described by Maniatis et al. (12). Restriction endonucleases and T4 DNA ligase were obtained from Bethesda Research Labora-

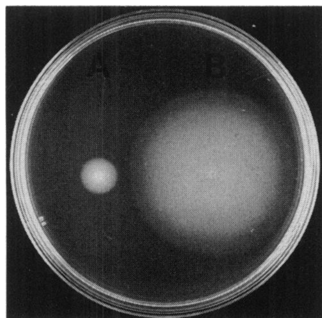

FIG. 1. Complementation of the *cheF* mutation by pGO102. *cheF* mutants containing either pSI-1 (A) or pGO102 (B) were spotted onto a tryptone swarm plate supplemented with 5 μg of chloramphenicol per ml.

tories, Inc. (Gaithersburg, Md.) and used as recommended by the supplier.

DNA sequencing techniques. M13mp18, M13mp19 (30), and phagemids pTZ18U and pTZ19U (13, 14) were used as vectors in the sequencing study. The nucleotide sequences of inserts in recombinant clones were determined by the dideoxynucleotide chain termination method of Sanger et al. (22). Sequenase (U.S. Biochemical Corp.) was used for the chain elongation in sequencing reactions. A 15-mer universal primer for sequencing was purchased from Bethesda Research Laboratories. [^{35}S]dATP was supplied by Amersham Corp. (Arlington Heights, Ill.). Other chemicals and nucleotides for the sequencing reaction were purchased as a kit from U.S. Biochemical.

RESULTS

Subcloning of the *cheF* gene. The plasmid DNA of pGO101 was digested with *Pst*I, which liberated five DNA fragments. Inserts were then ligated with linearized pSI-1. Competent cells of OI1925 were transformed with these ligated DNA mixtures. Transformants were selected, and the swarm phenotype of these transformants was screened. The swarm rate of *cheF* mutants was shown to be very slow compared with that of wild-type strains (20). Che$^+$ transformants were identified (Fig. 1) on the basis of their wild-type swarm phenotype, and plasmid DNAs were prepared from these cells. All Che$^+$ transformants carried a 700-base-pair *Pst*I fragment cloned in pSI-1. This pSI-1 derivative was designated pGO102 (Fig. 2). Cells containing pSI-1 did not convert their Che$^-$ phenotype to Che$^+$. To distinguish whether the Che$^+$ phenotype of the transformants resulted from pGO102 complementation or from reversion of *cheF141*, the following tests were performed. Plasmids were segregated from the Che$^+$ transformants by growing the transformants in the absence of chloramphenicol for a period of time. It

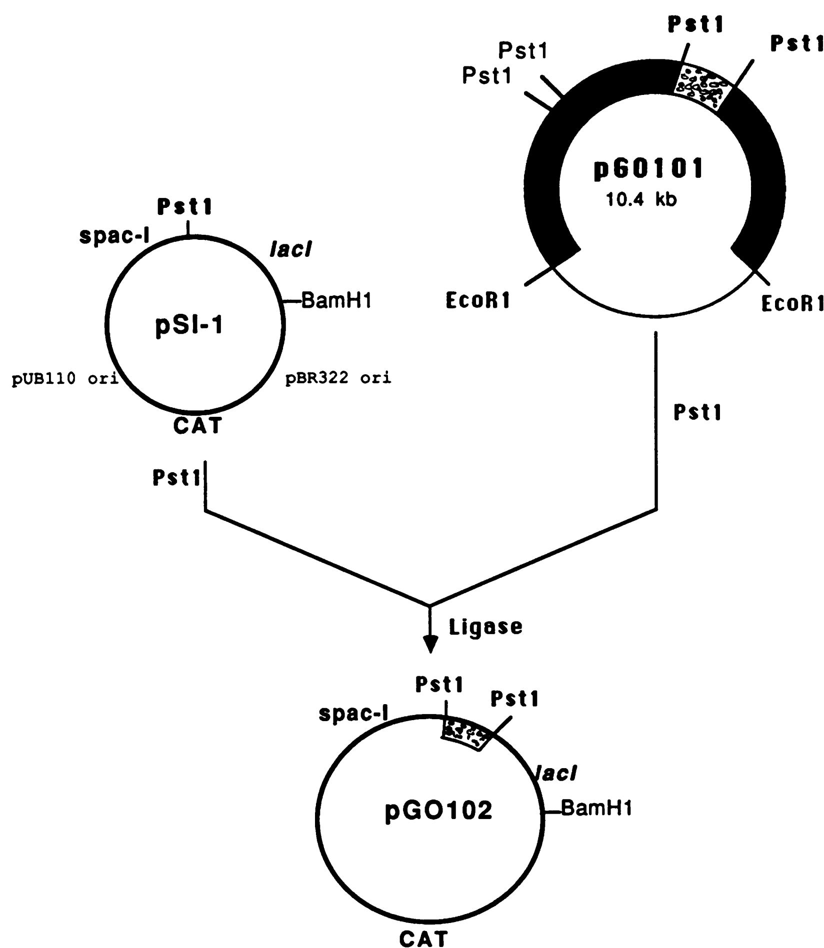

FIG. 2. Construction of pGO102. A 700-base-pair *Pst*I fragment (dotted box of pGO101) was inserted into the *Pst*I site of pSI-1. The solid box of pGO101 represents the 7.7-kilobase *B. subtilis* chromosomal DNA in a pUC18 vector, which is shown as a thin line.

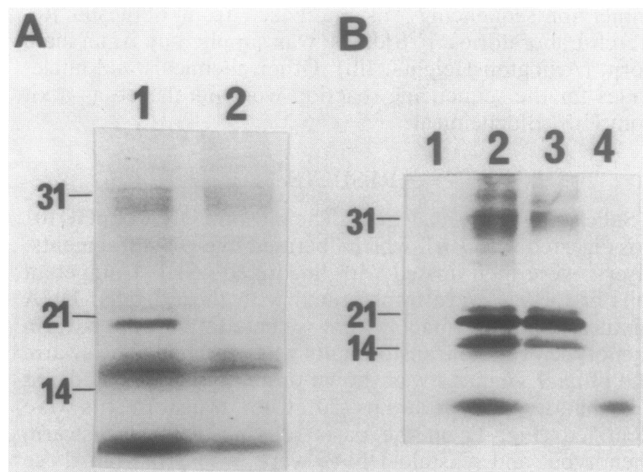

FIG. 3. Expression and localization of the *cheF* gene product. (A) Plasmid-encoded polypeptides of pGO102 (lane 1) and pSI-1 (lane 2) were expressed in *B. subtilis* maxicells. Strains containing either pGO102 or pSI-1 were UV irradiated and labeled with [^{35}S] methionine. Proteins were fractionated in a 12.5% sodium dodecyl sulfate-polyacrylamide gel and visualized by autoradiography. A 250-ng amount of protein was loaded on each lane. Molecular mass standards (in kilodaltons) are shown at the left. (B) The *cheF* protein was labeled by UV programming. Extracts were separated as the cytoplasm (lane 1) and the membrane (lane 2) fractions by ultracentrifugation. The membrane fraction of lane 2 was then washed with 1 M NaCl and centrifuged. After this centrifugation, the *cheF* protein appeared in the supernatant fraction (lane 3) and was removed from the membrane fraction (lane 4). Methods for separating each fraction are described in Materials and Methods. Approximately 350 ng of protein was loaded on each lane; molecular mass standards (in kilodaltons) are shown at the left.

was found that these cells regained the swarm phenotype, which was indistinguishable from that of *cheF* mutants. OI1925 was retransformed with pGO102 plasmid DNA, and the swarm phenotype of transformants was examined. All transformants checked were Che$^+$. To further confirm that this *Pst*I DNA fragment could complement *cheF* mutations, another chemotaxis assay was used. Chemotaxis of OI1925 and of OI1929, which contained pGO102, was analyzed by the capillary assay. In this assay, both OI1925 and OI1929 were grown in the presence of 1 mM IPTG (isopropyl-β-D-galactopyranoside) to induce the expression of plasmid-encoded polypeptides (32). OI1925 showed very poor taxis to the attractant aspartate in the capillary assay (20). The relative accumulation of OI1925 for 10^{-2} M aspartate was 1.49. OI1929, on the other hand, responded to the concentration gradient of aspartate as well as did the wild type. OI1925 and OI1878 (wild type) showed relative accumulation values of 11.5 and 10.9, respectively, for the same concentration of aspartate.

Identification and localization of the *cheF* gene product by using *B. subtilis* maxicells. We had established that the *cheF* structural gene was contained in pGO102. To determine the size of the *cheF* protein, the plasmid-encoded polypeptides were expressed from pGO102 in vivo by using *B. subtilis* maxicells (Fig. 3). Strains OI1925, OI1933, and OI1929 were UV irradiated, and newly synthesized proteins were labeled with [^{35}S]methionine as described in Materials and Methods. One predominant protein band unique to pGO102 was observed at a molecular weight of 20 kilodaltons (Fig. 3A). A second faint, slightly larger protein band was also observed; it is possible that this band represented a modified form of the major protein.

TABLE 2. Chemotaxis at various levels of *cheF* gene product

Strain	IPTG concn (mM)	Swarming rate (cm/h)	Relative accumulation[a]	% Increase in methanol production[b]
OI1929	0	0.038	3.0	30
	0.01	0.104	3.14	ND[c]
	0.1	0.092	6.9	68
	0.75	0.142	ND	105
	1.0	0.107	21.5	86
	10.0	0.106	13.4	35
	100.0	0.105	3.0	ND
OI1925	0	0.02	0.7	10
OI1878	0	0.24	23.1	125

[a] Expressed as number of cells accumulated in the capillary tube containing 3×10^{-5} M mannitol/number accumulated in the tube containing buffer. Each value is the mean of six replications.
[b] Amount generated upon addition of 2.3×10^{-2} M aspartate versus amount generated when buffer was added.
[c] ND, Not determined.

To localize the *cheF* protein, labeled maxicells were ruptured and fractionated as membrane, membrane-associated, and cytoplasm fractions by ultracentrifugation. The *cheF* gene product behaved like a membrane-associated protein, since it was removed from the membrane fraction after a salt wash of membranes (Fig. 3B).

Expression level of the *cheF* product for chemotaxis. To study the effect of altered *cheF* expression on chemotaxis, the gene was placed under the control of the spac-I promoter, which could be regulated by the addition of an inducer, IPTG. The level of the *cheF* gene product was altered in vivo by exposing the cells to various concentrations of IPTG in swarm plates.

In the absence of the inducer, a slow swarming rate was conferred by the plasmid (Table 2). The swarming rate of the cells reached a maximum at 0.75 mM IPTG and then decreased. As expected, IPTG did not affect swarming of the wild type (OI1878). Therefore, the swarming rate was inhibited by overproduction of the *cheF* protein.

To further investigate the effect of an elevated dosage of the *cheF* protein on behavior, the capillary assay was used. Cells were grown at various IPTG concentrations and then washed with buffer before the assay was performed. Bacteria responded to mannitol poorly when they were grown in a medium without IPTG (Table 2). They responded best when grown in 1 mM IPTG. The maximal accumulation was the same as that achieved by a wild-type control, OI1878. It was observed that a high IPTG concentration in the medium impaired chemotaxis of OI1929. The expression level of *cheF* showed a similar effect in capillary assays for aspartate (data not shown).

The effect of altered *cheF* expression levels on methanol production was explored. Labeled cells which had been grown in the presence of different IPTG concentrations were mixed with aspartate in Conway diffusion cells. The methanol released from cells as a result of aspartate stimulation was quantitated (Table 2). The optimal expression level of the *cheF* gene product for methanol formation occurred at 0.75 mM IPTG. The methanol evolved at this IPTG concentration was 80% of that evolved from the wild type (OI1878). The effect of elevated *cheF* protein dosage on methanol production was similar to that for accumulation in the capillary assay.

Nucleotide sequence of the *cheF* gene. The *Pst*I DNA

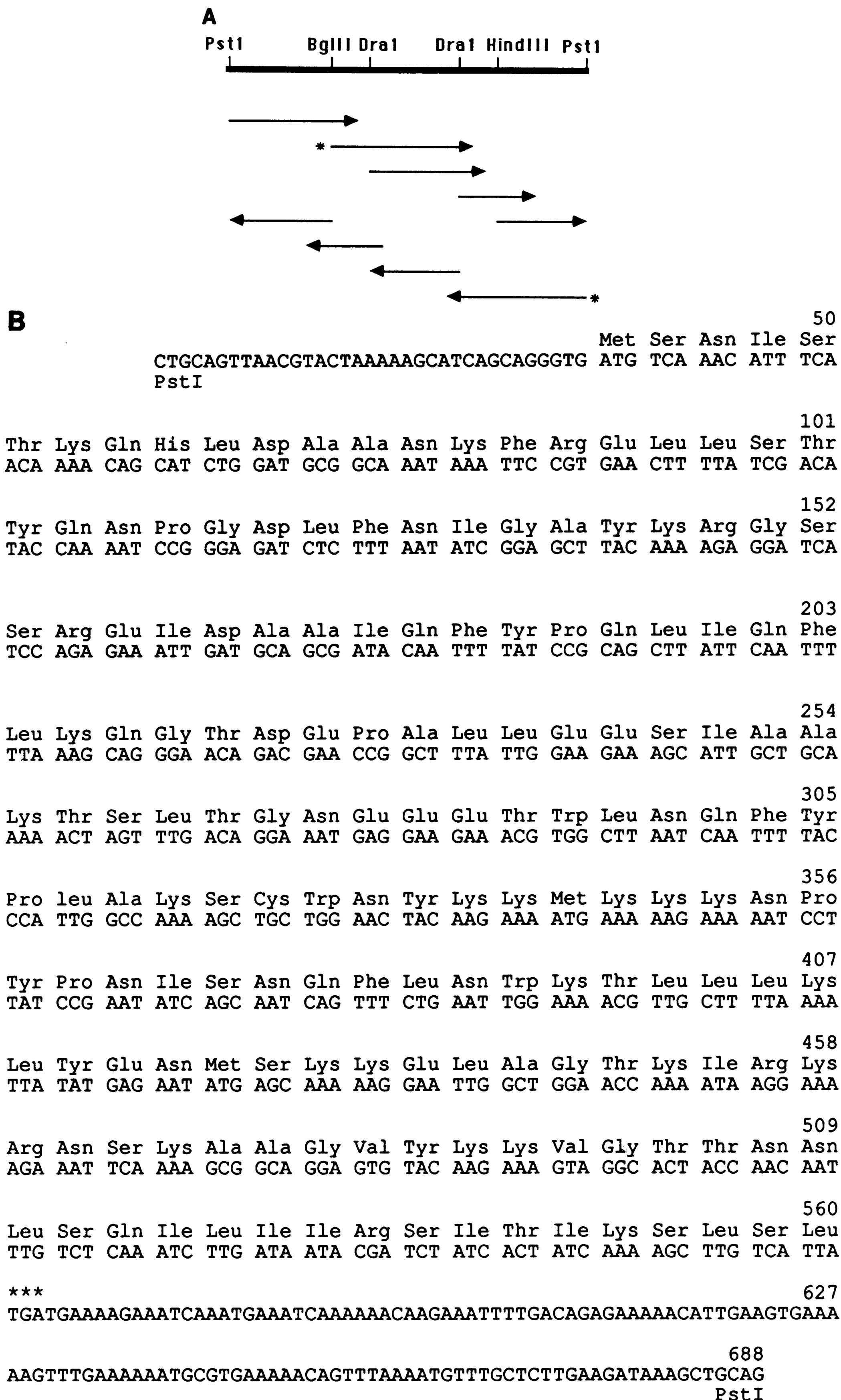

FIG. 4. Sequencing strategy for the 700-base-pair *PstI* fragment and nucleotide sequence of the *cheF* gene. (A) Restriction sites used in the sequencing experiments. All fragments except two inserts (*) were constructed in M13 phages; phagemids were used as the vectors for these two fragments. (B) Nucleotide sequence. Nucleotides are numbered by taking the position of the first nucleotide of *PstI* site as +1. Three asterisks mark the proposed translational stop codon.

fragment containing the *cheF* gene was sequenced (Fig. 4). Computer analysis of the DNA sequence revealed an open reading frame (ORF) of 528 bases (Fig. 4B). There are two potential initiation codons at the 5′ end of this ORF. These two codons are each in frame with the reading frame identified for the *cheF* gene. A potential ribosome-binding site is located in the nine-base region of the ATG codon at position 36. Six of seven bases (AGXAGGG) about six bases before the ATG codon are complementary to the 3′-OH end of 16S rRNA (8). On the basis of the rules of Tinoco et al. (27) and Borer et al. (1), the calculated ΔG of this interaction is -14.2 kcal (ca. -59.4 KJ)/mol. The GTG codon at position 33, however, is not preceded by a recognizable ribosome-binding site. Therefore, the ATG codon appears more likely to be the translational initiation codon. This ORF is capable of encoding a 175-amino-acid polypeptide with a calculated molecular weight of 20,002, in agreement with the molecular weight for the polypeptide expressed from pGO102 in maxicell experiments. To further confirm that this ORF encodes the functional *cheF* protein, mutations were generated by shifting the reading frame of the presumed coding region. pGO102 was digested with *Bgl*II, which is located within the putative ORF. Klenow fragment was used to either remove or fill in the sticky ends. The two new hybrid plasmids were then introduced into OI1925. No complementation by either of the plasmids was observed. Furthermore, in-frame translational fusion of the *cheF* protein to β-galactosidase was successfully constructed. Western blot (immunoblot) experiments confirmed the fusion, and the fused protein was purified (C. Ying and G. Ordal, manuscript in preparation).

The deduced amino acid sequence of the *cheF* gene product appeared to include a large group of lysine residues, 21 lysines in 175 amino acids; a pI value for the *cheF* protein of 9.81 was calculated. The hydropathy profile of the deduced *cheF* amino acid sequence (11) suggests that the *cheF* protein is composed of two quite different portions. Most of its sequence is characterized by long stretches of hydrophilic residues, whereas its carboxyl-terminal end contains a short hydrophobic region (^{159}LSQILIIRSITIKSLSL^{175}). Since it was shown that the *cheF* protein appears to be membrane associated, there are two possible means of interaction between the *cheF* protein and the membrane: the *cheF* protein might contact the membrane through its hydrophobic carboxyl terminus or could use its lysines to bind to the anionic residues of other membrane proteins.

DISCUSSION

This paper reports the first nucleotide sequence of a gene that is required for chemotaxis in *B. subtilis*. The *cheF* gene cloned on an expression plasmid complements the *cheF* mutation both for the chemotactic deficiency and for normal methanol production in response to the addition of attractants. Moreover, chemotaxis and methanol formation resulting from the stimulation of attractants both reach optimal levels when the *cheF* gene product is induced by about 0.75 mM IPTG. These results give evidence of a close relationship between methanol production and chemotaxis in *B. subtilis*.

Analysis of the nucleotide sequence of the *cheF* gene suggests that this gene is capable of encoding a protein with a molecular mass of 20 kilodaltons, in agreement with the finding of a protein of 20 kilodaltons in *B. subtilis* maxicells. The *cheF* gene is probably expressed at low levels in vivo, as indicated from the codon usage of the gene (7). The potential ribosome-binding site of the *cheF* gene has a lower than average calculated free energy of interaction with the 3′ end of 16S RNA. Taken together, these results imply that only low levels of *cheF* proteins are required for cells to perform chemotaxis normally. When the *cheF* product is overexpressed in the presence of high concentrations of IPTG, chemotaxis and methanol production are impaired. There are more than 20 other chemotaxis proteins, and the *cheF* protein may titrate one of them in causing this inhibition.

The hydropathy profile of the *cheF* protein indicates that the C-terminal part of the protein has a hydrophobic region. This region is too short to be membrane spanning, and our results suggest that the *cheF* protein is membrane associated under normal conditions but can be removed from the membrane by a high-salt wash. The *cheF* protein has a calculated pI of 9.81, and the basic nature of this protein is apparent from its amino acid sequence. The only other proteins besides MCPs involved in chemotaxis in *B. subtilis* that have been characterized are the methyltransferase and methylesterase. These proteins methylate and demethylate, respectively, MCPs in vitro. Both of these proteins are also basic and adhere to membranes (2, 5). It remains to be determined how the *cheF* protein and these two proteins interact with the membrane.

The deduced amino acid sequence of the *cheF* protein was compared with the sequences of other proteins in GenBank (E. Kofoid and J. S. Parkinson, personal communication). No significant homology between the *cheF* protein and any known proteins was observed. *cheF* mutants are impaired in release of methanol caused by addition of attractant or removal of repellent (20). There is strong evidence that methyl groups are not released directly from the *B. subtilis* MCPs but instead are transferred (as methyl or methoxy groups) to some other carrier(s) (26, 26a). Therefore, it is tempting to speculate that the actual substrate is one of these methyl carriers. It is clear that the *cheF* product functions in a pathway unique in *B. subtilis*, one not found in *E. coli*.

Determination of the substrate for the *cheF* protein and elucidation of the mechanism by which it associates with the membrane will be possible after the protein has been purified. We have already been able to express the protein at a relatively high level under the control of the spac-I promoter, and we are attempting to raise antibodies against the *cheF* protein to aid in this process of analyzing the role of this protein in the molecular mechanism of chemotaxis.

ACKNOWLEDGMENTS

We thank D. Henner for the gift of pSI-1 and J. Kaput for valuable suggestions during the course of this investigation. We also thank Aamir Zuberi for help in preparation of the manuscript. Comparison of the deduced *cheF* protein with proteins in GenBank was performed by Eric Kofoid in the laboratory of J. S. Parkinson.

This work was supported by Public Health Service grant AI 0336 from the National Institutes of Health and National Science Foundation grant DCB 85-01604.

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