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Characterization of a Methyl-Accepting Chemotaxis Protein Gene, *dmcA*, from the Oral Spirochete *Treponema denticola*

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A gene, *dmcA*, expressing a methyl-accepting chemotaxis protein (MCP) from the oral spirochete *Treponema denticola* has been characterized. The gene was initially identified as an open reading frame immediately upstream from the previously characterized *prtB* protease gene of strain ATCC 35405. Nucleotide sequencing of the *dmcA* gene revealed a potential 57-kDa protein product with extensive homology with the signaling regions of MCPs from a variety of bacteria. The protein expressed in *Escherichia coli* cross-reacted with anti-Trg (*E. coli* MCP) serum, confirming its homology with MCPs. Northern blot and primer extension analyses identified the transcription start site of the monocistronic *dmcA* mRNA. By utilizing a *T. denticola* gene inactivation system recently developed in this laboratory, a mutant defective in the *dmcA* gene, HL0501, was constructed. The mutant was demonstrated to be defective in chemotaxis toward nutrients. In addition, the methylation profiles of cellular proteins indicated altered MCPs in the mutant relative to those of the parental strain. These results indicate that we have identified an MCP gene in the oral spirochete which plays a significant role in the chemotactic response of the organism.

Human periodontal diseases are generally characterized as mixed bacterial infections (27). One of the predominant groups of microorganisms identified in inflamed periodontal sites is the spirochetes (15). Many of these organisms cannot be cultivated in the laboratory, but one of them, *Treponema denticola* (20), can be readily grown in vitro and has been positively correlated with the severity of periodontitis (26). Like other spirochetes, *T. denticola* expresses periplasmic flagella which are involved in the characteristic motility of these organisms (6). Although genes involved in the flagellar structure of spirochetes have been isolated recently (14), methyl-accepting chemotaxis protein (MCP) homologs from these organisms have yet to be characterized. Recently, a form of chemotaxis has been demonstrated for *T. denticola* (5), but the nature of the effectors involved has not yet been determined. Such motility may be an important virulence property allowing the organisms to penetrate periodontal tissue in order to migrate to environmentally favorable sites for proliferation (15).

Among the potential virulence factors expressed by *T. denticola*, proteases appear to be important in pathogenicity (15). As a result of isolating and characterizing a chymotrypsinlike protease gene, *prtB*, from *T. denticola* ATCC 35405 in this laboratory (2), we identified an open reading frame (ORF) immediately upstream of the *prtB* gene. Primer extension analysis of the *prtB* gene indicated that ORF1 was not part of the *prtB* operon. Since the carboxyl-terminal domain of the putative ORF1 gene product displayed extensive homology with the signaling regions of MCPs, it was of interest to further characterize this gene. The MCPs play important regulatory roles in modulating both attraction and repulsion in motile bacteria (12), and the corresponding genes for these proteins have not yet been isolated from oral spirochetes. The presence of MCPs in spirochetes has been suggested on the basis of immunological analysis (17). In addition, a *T. denticola* MCP should play a significant role in regulating the reported invasive properties of these organisms in periodontally inflamed tissue (15). The present communication describes the isolation and characterization of a gene, *dmcA* (*denticola* methyl-accepting chemotaxis A), located immediately upstream of the *prtB* gene on the *T. denticola* ATCC 35405 chromosome.

MATERIALS AND METHODS

Bacterial strains and cultivation. *T. denticola* ATCC 35405, which was previously isolated from a human periodontal pocket (6a), was maintained and grown at 37°C in an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, Mich.) under 85% N_2, 10% H_2, and 5% CO_2 in TYGVS broth medium (19). *Escherichia coli* JM109 was used as a host strain for pBluescript II SK+/− phagemid (Stratagene, La Jolla, Calif.) and was routinely grown at 30°C in Luria broth (LB) (22) or on LB agar plates. *E. coli* RP8611 (14a) (Δ*tsr-7028* Δ*tar-tap 5201* Δ*trg-100*) was kindly provided by J. S. Parkinson (University of Utah, Salt Lake City). Transformed cells were grown in LB supplemented with ampicillin (100 μg/ml).

Western blot analysis. For detection of MCPs in *T. denticola*, cell extracts were analyzed on Western blots using rabbit anti-Trg serum (17) (provided by R. Hazelbauer, Washington State University, Pullman). A 3-day culture of strain ATCC 35405 was harvested from 20 ml of TYGVS broth, washed with PBS (0.15 M NaCl, 10 mM potassium phosphate buffer; pH 7.2), and resuspended in 1.0 ml of 0.05 M Tris-HCl buffer, pH 7.5. The cells were also disrupted following ultrasonication (100 W for 3 min) in a model 450 sonifier (Branson, Danbury, Conn.). After removal of the cell debris following centrifugation at 12,000 × *g* for 30 min, the supernatant fluids were used as the cell extracts. Samples were boiled for 2 min in electrophoresis buffer (2% sodium dodecyl sulfate [SDS], 5% mercaptoethanol, 10% glycerol, 0.0005% bromophenol blue, 0.0625% Tris-HCl; pH 6.8) and applied to an SDS–10% polyacrylamide gel run at 100 V (12a) for 2 h. Proteins were electroblotted with a transblotter (Bio-Rad Laboratories, Richmond, Calif.) at 70 V for 25 min in 10 mM CAPS [3-(cyclohexylamino)-1-propanesulfonic acid]–10% (vol/vol) methanol (pH 11.0) to Immobilon polyvinylidene difluoride membranes (Millipore Co., Bedford, Mass.). Following protein transfer, the Immun-Blot horseradish peroxidase assay system (Bio-Rad) was used to detect the proteins reactive with the indicated serum according to the instructions of the supplier.

For detection of *T. denticola* proteins expressed in *E. coli* which cross-reacted with anti-Trg serum, strain RP8611 or JM109 carrying the recombinant plasmids was grown on LB agar plates plus ampicillin plus 0.05 mM IPTG (isopropyl thio-β-galactoside) for 3 days at room temperature since the plasmids were unstable when present in cells grown in liquid culture. The cells were harvested, resuspended in 50 mM Tris buffer (pH 7.5), and sonicated, and the extracts were employed for Western blotting as described above.

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IPCR for isolation of the 5′ end of the *dmcA* gene. In order to isolate the 5′ end of the *dmcA* gene, which was previously identified as an ORF upstream from the *prtB* gene (2), an inverse PCR (IPCR) procedure was carried out as previously described by Ochman et al. (18). Chromosomal DNA from strain ATCC 35405 was completely digested with *Taq*I, *Sau*3AI, and *Alu*I, and each digested DNA was self-ligated with T4 DNA ligase. The ligated DNA samples were utilized as templates for IPCR. For each reaction, two 21-mer oligonucleotide primers (5′-CAGTTCTTGCCCCGTCTTTAC-3′ [nucleotides 1294 to 1274] and 5′-TTGAAGCGAGCCAAATCATTC-3′ [nucleotides 1340 to 1360] for *Taq*I, 5′-CGTTGATACTTTCGGTCTGCA-3′ [nucleotides 1165 to 1145] and 5′-TTACGGCCATGGCGGAAAACA-3′ [nucleotides 1181 to 1201] for *Sau*3AI, and 5′-AGTCCAGACCTATACAGCCTA-3′ [nucleotides 400 to 380] and 5′-TTTAGCCGACCCAAAACATGC-3′ [nucleotides 489 to 509] for *Alu*I) synthesized by the oligonucleotide synthesis facility of the Department of Biochemical Pharmacology, State University of New York, Buffalo, were used together with Vent DNA polymerase (New England Biolabs, Inc., Beverly, Mass.). The primer sequences were chosen on the basis of the presence of the three restriction sites near the 5′ end of the previously sequenced portion of the *dmcA* gene. Conditions for IPCR and PCR were denaturation for 1 min at 94°C, annealing for 2 min at 55°C, and extension for 3 min at 72°C for 25 cycles.

Isolation of nucleic acids. Chromosomal DNA from *T. denticola* was isolated by the method of Sambrook et al. (22). Total RNA was extracted from cells grown in 100 ml of TYGVS medium by the boiling method (25). Briefly, the cells were harvested after growth for 3 days and suspended in 4 ml of Tris-EDTA containing 5 mg of lysozyme/ml. After standing for 5 min on ice, the suspensions were treated with 400 μl of 10% SDS and boiled for 2 min. Cellular debris was removed by centrifugation, and the supernatant fluids were extracted with 2 ml of water-saturated phenol and subsequently with 2 ml of chloroform. After ethanol precipitation following addition of 200 μl of 3 M sodium acetate and 10 ml of ethanol, the RNA was dissolved in 100 μl of diethyl pyrocarbonate (Sigma, St. Louis, Mo.)-treated distilled water.

Southern blot analysis. Chromosomal DNA was digested with the indicated enzymes, separated on 1.0% agarose gels, and transferred to Hybond-N^+ nylon membranes (Amersham International plc., Amersham, United Kingdom) by capillary transfer (22). Enhanced chemiluminescence (ECL) direct nucleic acid labeling and detection systems (Amersham) were used to identify the DNA fragments with the indicated probes. Hybridization was performed at 42°C overnight, and the hybridized membranes were washed in 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 6 M urea and 0.4% SDS at 42°C as well as at room temperature as specified by the supplier. The blots were then exposed to blue-light-sensitive autoradiography film (Hyperfilm-ECL; Amersham) for 1 min.

Northern blot analysis. Total RNA isolated from *T. denticola* was separated by electrophoresis on a 2.2 M formaldehyde–1.2% agarose gel and transferred to Hybond-N^+ nylon membranes by capillary transfer. The ECL direct nucleic acid labeling and detection systems (Amersham) were used to detect hybridizing bands as outlined above for Southern blot analysis with the indicated probes. The blots were exposed to blue-light-sensitive autoradiography film for 30 min.

Nucleotide sequencing of the *dmcA* gene. The nucleotide sequence of the *dmcA* gene was determined from both DNA strands by using the dideoxynucleotide sequencing strategy (23). Each DNA fragment from the IPCRs was subcloned into pBluescript II SK+ or KS+. Single-stranded template DNA was isolated by the method of Messing (16) utilizing M13K07 helper phage (Bio-Rad). Sequencing was carried out with Sequenase 2.0 (United States Biochemical Corp., Cleveland, Ohio) and T7 or synthetic oligonucleotide primers with ^{35}S-dATP (Dupont NEN Research Products, Wilmington, Del.). Sequence analysis was performed with IBI Pustell (International Biotechnologies, Inc., New Haven, Conn.) and HIBIO DNASIS (Hitachi Software Engineering Co., Ltd., Japan) sequence analysis programs.

Primer extension analysis. A 21-mer oligonucleotide primer (5′-TCCGTCGTTTTGCATAAGCAT-3′) synthesized by the State University of New York oligonucleotide sequencing facility was labeled with [γ-^{32}P]ATP (Dupont) and used as a primer for each analysis. This oligonucleotide anneals to nucleotides 463 to 483 within the *dmcA* sequence. Reverse transcriptase (GIBCO Bethesda Research Laboratories, Gaithersburg, Md.) was used to extend each primer to produce cDNA complementary to *T. denticola* mRNA following annealing. After RNase A treatment, the resulting end-labeled DNA was electrophoresed on 12% polyacrylamide gels (Long Ranger; AT Biochemicals, Inc., Malvern, Pa.) under denaturing conditions. Dideoxynucleotide sequencing reaction mixtures with the same oligonucleotide primers were electrophoresed in parallel. The dried gels were then exposed to Kodak XAR-2 (X-Omat AR) film.

Expression of *dmcA* in *E. coli*. In order to express the *dmcA* gene in *E. coli*, a *lacZ′-dmcA* fusion was constructed so that the N-terminal amino acids of β-galactosidase were fused in frame to the *dmcA* gene lacking approximately 20% of the N-terminal sequence. Two 29-mer oligonucleotide primers, 5′-GGGGATCCCTATTGTATGCTTATGCAA-3′ (which binds to nucleotides 456 to 479) and 5′-GGGAATTCTATTCTTTTAATGGCATCA-3′ (corresponding to nucleotides 1678 to 1696), containing the *Bam*HI and *Eco*RI sites, respectively, were used as primers for PCR together with *T. denticola* chromosomal DNA as the template to isolate the 1,240-bp DNA fragment coding for the *dmcA* gene. The PCR product was cleaved with *Bam*HI and *Eco*RI, ligated with similarly cleaved pUC19, and transformed into *E. coli* RP8611.

Construction of a *dmcA* mutant. An insertionally inactivated *dmcA* mutant of strain ATCC 35405 was constructed by a procedure recently utilized to construct a *flgE* mutant of this strain (13). Briefly, an 838-bp internal *Ssp*I fragment of the *dmcA* gene in plasmid pUC01 was subcloned into the *Pvu*II site of plasmid pKmOZ18 (25) to obtain plasmid pKmdmcA. This plasmid was then cleaved within the *dmcA* gene with *Pvu*II, and an erythromycin resistance (Em^r) cassette (13) was inserted to produce plasmid pHLdmcA. The linearized plasmid (*Xho*I digested) was utilized to transform *T. denticola* by electroporation as described previously (13). The Em^r transformants were isolated, grown in TYGVS-erythromycin broth to log phase, and analyzed as described above.

Chemotaxis assays. The three-layer chemotaxis migration system (5) was modified to investigate cell migration toward chemoattractants. Briefly, the top and bottom layers consisted of TYGVS-agarose medium containing 10% rabbit serum and 10% nutrient supplements (5), while the middle layer contained only TYGVS-agarose medium. The designations top (neck end) and bottom have reference only to the geometry of the tubes, since the tubes were incubated horizontally. Approximately 10^8 cells were used to inoculate the middle layers before solidification. To minimize the potential contributions of other chemoeffectors, such as the anaerobic gases, the tubes were filled to the tops and the caps were tightened during incubation. The tubes were incubated horizontally for 12 days in the anaerobic chamber before the results were documented by photography.

Methylation assays. The wild-type and *dmcA* mutant cells were grown to late exponential growth phase, and approximately 2×10^9 cells were harvested and washed with chemotaxis buffer (10 mM potassium phosphate, 0.2 mM L-cysteine hydrochloride; pH 7.0). The cells were next suspended in 100 μl of chemotaxis buffer containing chloramphenicol (100 μg/ml) and 2 μM L-[*methyl*-^{3}H]methionine (15 to 45 μCi/ml; Amersham). After 30 min of aerobic basal methylation, chemoattractants (10% rabbit serum and 10% nutrient supplements [13]) were added to the cell suspension, and the mixture was incubated for an additional 30 min at 37°C. The reactions were stopped by centrifugation (20,000 × *g* for 3 min), and the cell pellets were resuspended in 80 μl of electrophoresis sample buffer and boiled for 5 min. A special protein methylation SDS-polyacrylamide gel electrophoresis system (24) was utilized, and protein equivalent to 2×10^8 cells was loaded into each well. Electrophoresis was done at 50 mV until the dye front reached the bottom of the gel, and the gel was then stained with Coomassie blue. After being destained, the gel was immersed in fixing solution (5% acetic acid–5% isopropyl alcohol) for 20 min. Fluorography was carried out by immersing the gel in an Autofluor Autoradiographic Image Intensifier (National Diagnostics, Atlanta, Ga.) for 45 min with gentle agitation. The gel was then transferred to 3MM filter paper and vacuum dried. The radiolabeled methylated-protein bands were detected following exposure of the gel to BioMax Kodak Scientific Imaging film (Eastman Kodak Company, Rochester, N.Y.) at −70°C for 6 days.

Nucleotide sequence accession number. The nucleotide sequence of the *dmcA* gene has been deposited in the GenBank database under accession no. U33210.

RESULTS

Isolation of the *dmcA* gene. Previously, the *prtB* gene coding for a *T. denticola* chymotrypsinlike protease was identified in this laboratory (2). Sequencing upstream of this gene revealed an ORF (ORF1) whose deduced carboxyl-terminal region exhibited extensive homology with bacterial MCPs. Therefore, since the intact ORF1 was missing from the *T. denticola* insert in plasmid pSA2 (2) harboring *prtB*, an IPCR strategy was utilized to isolate the 5′ region of the gene. By utilizing fragments isolated from strain ATCC 35405 chromosomal DNA cleaved with *Taq*I, *Sau*3AI, and *Alu*I as templates, overlapping fragments containing the 5′ end of the gene were isolated following IPCR as described above.

Southern blot analysis (data not shown) confirmed that ORF1 was derived from the *T. denticola* chromosome. A DNA probe (*Kpn*I-*Hin*dIII fragment) comprising the 3′ end of the gene hybridized with single DNA fragments derived from strain ATCC 35405 chromosomal DNA cleaved with *Hin*dIII, *Pst*I, *Cla*I, and *Xho*I. These results are compatible with the known restriction map of this region previously determined for plasmid pSA2 (2).

Nucleotide sequencing of DNA fragments containing ORF1 identified a putative 1.5-kb gene which would code for a protein of 57 kDa (data not shown). Although the reading frame of ORF1 continued upstream of the presumed ATG initiation codon starting at nucleotide 136, primer extension analysis (see Fig. 3) suggested that this position represented the most logical start site for the gene. Since the sequence of the 3′ end of the

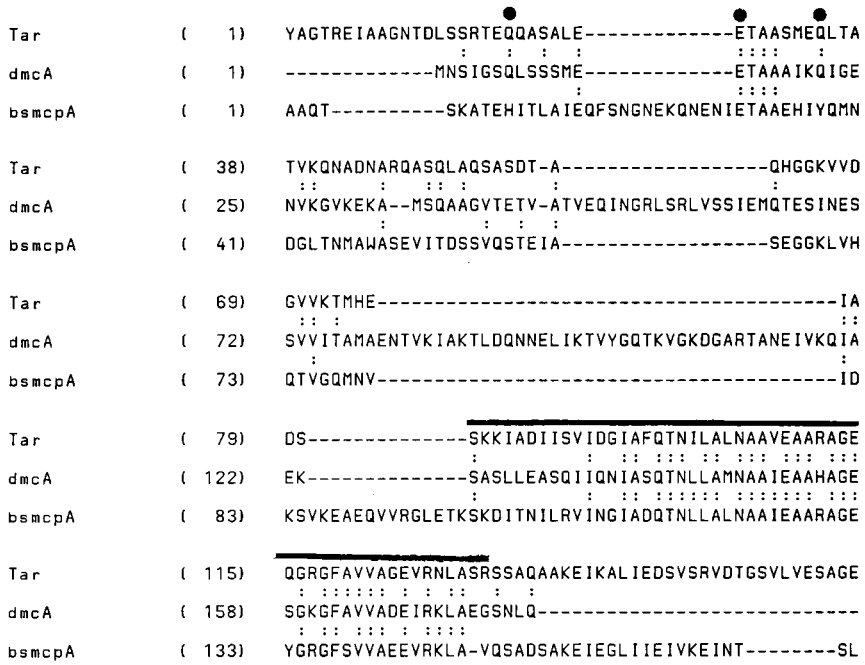

FIG. 1. Comparison of the DmcA sequence with other bacterial MCPs. Tar, *E. coli* Tar; *dmcA*, *T. denticola dmcA*; *bsmcp*A, *B. subtilis mcpA*. HCD (overline) and K1 methylation sites of Tar (solid circles) are indicated. Numbers at the left of each sequence represent the positions relative to the Tyr residue of Tar.

16S rRNA from *T. denticola* has not yet been determined, it is not possible to unequivocally identify the ribosome binding site for the gene. Promoter sequences homologous to those of alternate sigma factors known to be involved in the expression of chemotaxis genes (6) were not identified near the transcription start site. Consensus sequences for *T. denticola* promoters have not yet been established. In addition, no potential transcription terminator was apparent in the sequence determined downstream from the stop codon.

The deduced amino acid sequence of the ORF1 product revealed that the protein contained two hydrophobic potential membrane-spanning regions in the N-terminal half of the protein (amino acids 42 to 72 and 183 to 216). In addition, a comparison of the amino acid sequence with the NBRF database revealed that a portion of ORF1 exhibited extensive homology with the signaling domains of MCPs (Fig. 1). This was especially true for the highly conserved domain (HCD) regions. In addition, three amino acid positions corresponding to the methylation sites of the K1 region of gram-negative enteric MCP signaling domains (4) were conserved in ORF1. The overall sequence homology of ORF1 with the *E. coli* MCP Tar was 26% for a 365-amino-acid overlap, whereas it was 29% for the *Bacillus subtilis* McpA protein in a comparison of 513 amino acids (10).

Transcriptional analysis of ORF1. Northern blot analysis of the ORF1 transcript using a DNA probe from the 3′ end of ORF1 demonstrated two transcripts of 1.3 and 1.6 kb (Fig. 2). The larger transcript is compatible with the proposed size of the ORF1 gene of 1.5 kb. It is not clear if the smaller transcript represents altered initiation or termination of transcription of the gene. It is also possible that the latter transcript might represent interaction of the transcript with 16S rRNA. The size of the ORF1 mRNA suggests that this gene is not part of an operon structure, although its presence in a larger message cannot be formally excluded.

Primer extension analysis of ORF1 (Fig. 3) indicated that transcription was initiated at a G residue at position 67. On the basis of this position, a sequence (TATACA) similar to the *E. coli* −10 consensus sequence (TATAAT) could be identified, but no corresponding −35 sequence was apparent. However, the consensus structure of *T. denticola* promoters has yet to be defined.

Expression of ORF1 in *E. coli*. In order to characterize the protein product of ORF1, the gene was introduced into plasmid pUC19 under the control of the *lac* promoter. For this purpose, a DNA fragment containing ORF1 was isolated following PCR as a *Bam*HI-*Eco*RI fragment and introduced into pUC19 (see Materials and Methods). The construct contained the first 10 amino acids of β-galactosidase fused in frame to ORF1, beginning at amino acid 80. Induction of the fusion protein demonstrated the appearance of a protein band of approximately 48 kDa which reacted weakly with anti-Trg se-

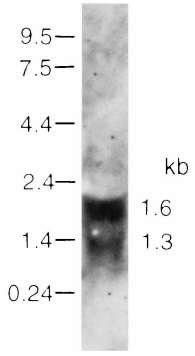

FIG. 2. Northern blot analysis of the *dmcA* mRNA. Total RNA from strain ATCC 35405 was hybridized with the labeled *Kpn*I-*Hin*dIII fragment from plasmid pSA2 as a probe. Molecular size markers (in kilobase pairs) are shown on the left.

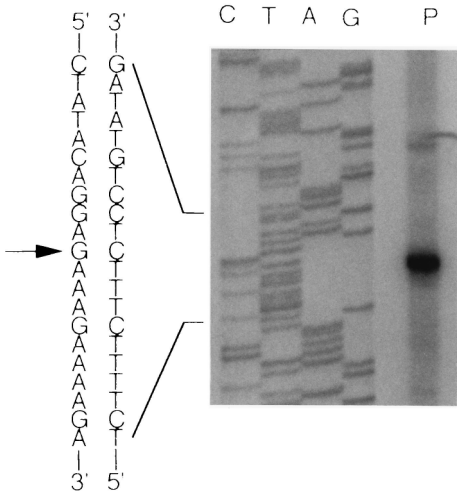

FIG. 3. Primer extension analysis of the *dmcA* gene. The position of the start site was determined in lane P (arrow) relative to the nucleotide sequence of the same region. A primer corresponding to positions 1048 to 1068 was utilized for both primer extension and nucleotide sequencing.

rum (data not shown). This apparent size is compatible with that predicted from the deduced amino acid sequence of ORF1 plus the amino acids from LacZ′. Antiserum against the *E. coli* MCP, Trg, has been demonstrated to cross-react with putative MCPs from a variety of bacterial species (17).

In order to examine directly the relationship between the MCPs and *T. denticola* proteins, Western blot analysis of strain ATCC 35405 proteins with anti-Trg serum was carried out. The results (Fig. 4) indicated that a strain ATCC 35405 cytoplasmic protein of approximately 65 kDa cross-reacted with the serum in the crude extracts. This size is somewhat larger than that deduced from the sequence of ORF1 and suggests that the protein product migrates more slowly than predicted on SDS-polyacrylamide gels. In addition, several other higher-molecular-mass bands were also visible. However, since multiple MCPs are present in most bacteria (10), it was not possible to precisely determine which band corresponded to the ORF1 product. Nevertheless, on the basis of these results as

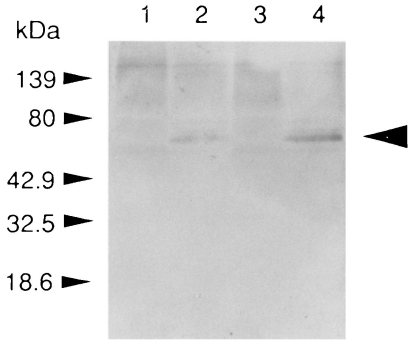

FIG. 4. Western blot analysis of MCPs in strain ATCC 35405 and the *dmcA* mutant. Cells were harvested in the late log phase and analyzed for proteins as described in the text following loading of the samples onto SDS–10% polyacrylamide gels. The protocol for stimulating the cells for methylation was utilized in the absence of methionine. Lanes: 1, *dmcA* mutant stimulated with nutrient supplements; 2, parental strain ATCC 35405 stimulated with supplements; 3, *dmcA* mutant without supplements; 4, parental cells without supplements. The DmcA protein which reacted with anti-Trg serum is indicated (arrowhead).

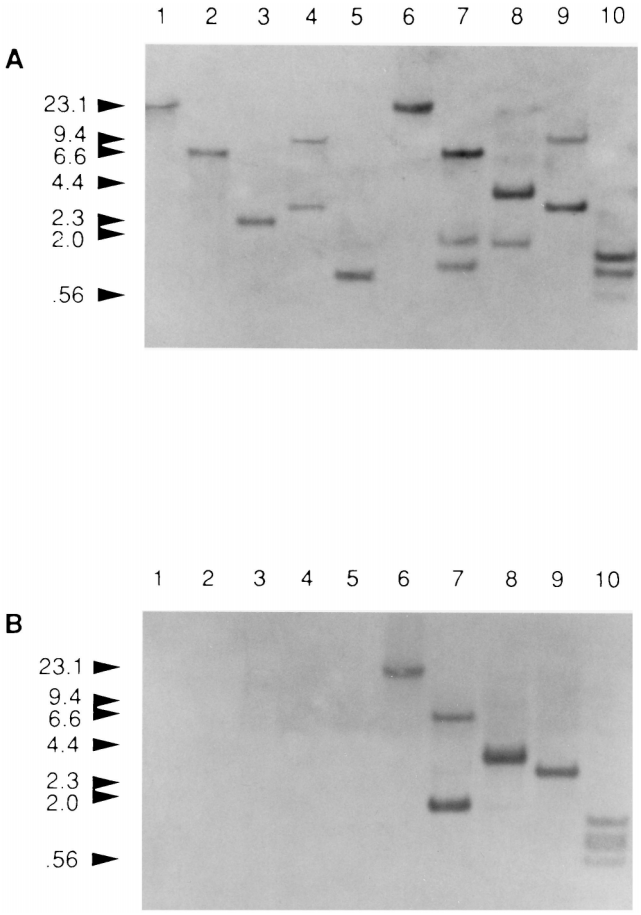

FIG. 5. Southern blot analysis of mutant HL0501. Lanes: 1 to 5, wild-type strain ATCC 35405; 6 to 10, mutant HL0501. Lanes 1 and 6, uncut chromosomal DNA; lanes 2 and 7, *Hin*dIII-cut DNA; lanes 3 and 8, *Pst*I-cleaved DNA; lanes 4 and 9, *Pvu*II-cut DNA; lanes 5 and 10, *Ssp*I-cut DNA. The probe for panel A was the 0.84-kb *Ssp*I *dmcA* gene fragment from pUC19/ORF1, while that for panel B was the 2.1-kb *Sst*I/*Pst*I fragment from the Em^r cassette. Molecular sizes (in kilobase pairs) are shown on the left.

well as those described below, the ORF1 gene was designated *dmcA*.

Properties of a *dmcA* mutant. In order to directly determine the function of the DmcA protein, a specific *dmcA* mutant was constructed. By utilizing a strategy recently developed in our laboratory for gene inactivation in *T. denticola* (13), the gene was interrupted with an Em^r cassette. Both Southern blot (Fig. 5) and PCR (data not shown) analyses confirmed the correct inactivation of the gene in the strain ATCC 35405 chromosome. The Em^r cassette probe did not react with any DNA fragments from the wild-type organism but did hybridize with those from the transformants (Fig. 5B). Likewise, the *dmcA* probe hybridized with the predicted-size bands from the transformants which were distinct from the fragments detected in the wild-type organism (Fig. 5A).

One representative transformant, HL0501, was shown to be defective in chemotaxis toward nutrient-containing media (Fig. 6) which had previously been demonstrated for these organisms (13). By use of either a three-phase or a two-phase (data not shown) system, it could be demonstrated that the wild-type strain ATCC 35405, but not mutant HL0501, migrated from the nutrient-poor layers into the agarose layers containing the nutrient supplements. However, the mutant appeared to be as

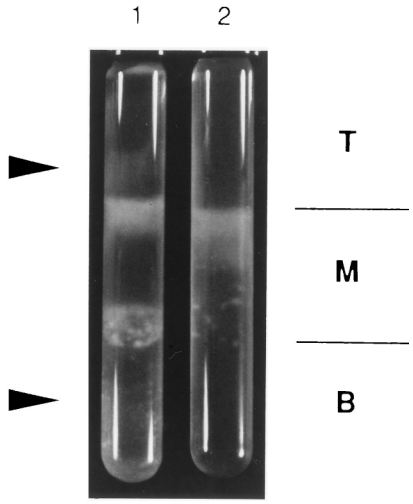

FIG. 6. Chemotactic behavior of the *dmcA* mutant. Following inoculation of the middle layer (TYGVS-agarose), migration of the cells into the top and bottom layers (TYGVS-agarose containing nutrients) was monitored. Tube 1, wild-type 35405; tube 2, mutant HL0501. The boundaries between the top (T), middle (M), and bottom (B) layers as described in the text (lines) and the migration of the wild-type strain into the top and bottom layers (arrowheads) are indicated.

motile as the parental organism when suspended in water and viewed under phase-contrast microscopy. This indicated that the mutant still expressed the periplasmic flagella required for motility. An analogous mutant in strain ATCC 33520 of *T. denticola* constructed by the same strategy also was altered in chemotaxis (data not shown). However, the repulsive behavior of the mutant toward agar-containing media (13) did not appear to be affected (data not shown). These results suggested that the *dmcA* gene is essential for chemoattraction toward certain nutrients.

If the *dmcA* gene codes for an MCP, it would be expected that the protein methylation pattern of mutant HL0501 would be altered relative to that of the parental organism. That this was indeed the case was demonstrated following such an analysis (Fig. 7). Methylation of proteins of approximately 65 and 35 to 43 kDa could be readily demonstrated in the wild-type

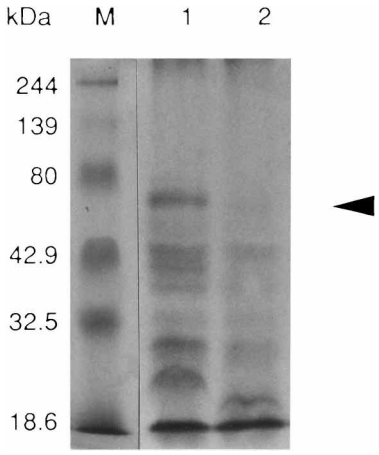

FIG. 7. Protein methylation patterns of the parental and *dmcA* mutant strains. Lanes: M, Sigma molecular mass markers; 1, strain ATCC 35405; 2, mutant HL0501. The proteins which appear to be undermethylated in the mutant strain are indicated (arrowhead).

strain in the presence of nutrient supplements. However, no methylation of the 65-kDa protein in mutant HL0501 was detected. In addition, much weaker methylation of the 35- to 43-kDa proteins also occurred in the mutant strain. Alterations in the methylated-protein patterns were also detected for lower-molecular-mass proteins, but it is not clear whether these represent distinct methylated proteins or breakdown products of larger proteins. These results suggested that the 65-kDa methylated protein is the product of the *dmcA* gene, confirming the results of Western blotting (Fig. 4). Taken together, these results are strongly indicative of the *dmcA* gene coding for an MCP in *T. denticola*.

DISCUSSION

The characteristic motility of the spirochetes is dependent upon the ability of these organisms to both sense and move toward or away from attractant or repellent molecules. Chemotaxis of these organisms is mediated by periplasmic flagella which, unlike in most other organisms, do not usually protrude from the exterior surface of the cells (6). For bacteria, the rotation of these organelles is regulated by interaction with the chemotaxis signaling systems associated with the cytoplasmic membranes (28). An essential component of these systems is the MCPs, which can interact either directly or indirectly with small molecules to sequentially signal the Che downstream proteins of the regulatory cascade. Immunological analyses have revealed the likely presence of MCPs in spirochetes (17). To our knowledge, the present communication reports the initial isolation and characterization of an MCP gene from an oral spirochete.

Nucleotide sequencing upstream from the 3′ end of the previously identified ORF1 revealed the presence of a gene encompassing this ORF, which we have termed *dmcA*. The apparent size of the DmcA protein, approximately 57 kDa, is slightly below the 60- to 70-kDa range for most bacterial MCPs (28). However, a recent immunological analysis utilizing anti-Trg serum has suggested that the homologous MCPs in spirochetes may be somewhat smaller than the proteins from most other bacteria (17). That this gene encodes an MCP was suggested by several different approaches. Part of the sequence of *dmcA* showed extensive sequence homology with the HCD present in all sequenced bacterial MCPs (28). Likewise, several conserved amino acid residues present in the K1 methylation region of the MCPs were shared with the *T. denticola* DmcA sequence. However, several proteins which originally were not thought to play a role in chemotaxis also contain sequences which are homologous to the HCD regions (1). Nevertheless, for one of these proteins, *Vibrio cholerae* HlyB, this has been reexamined (15a). For this reason, other approaches in addition to sequence homology are required to identify MCPs.

Western blot analysis utilizing anti-Trg serum has indicated that proteins showing immunological cross-reactivity with the *E. coli* MCP Trg are expressed in *T. denticola*. The major 65-kDa protein band which cross-reacted with anti-Trg serum also was lost following inactivation of the *dmcA* gene. Furthermore, the antiserum also cross-reacted with the fusion protein expressed from the *dmcA* gene in *E. coli*. Confirmation of the identity of the DmcA protein as an MCP was provided by functional analyses using a *dmcA* mutant constructed following electroporation. The mutant was directly demonstrated to be defective in its ability to display chemotaxis in a model chemoattraction system. Furthermore, methylation analysis indicated that the *dmcA* mutation altered the methylation patterns of proteins in *T. denticola*. Although it is difficult to unequivocally identify individual MCP species from gel analysis due to

the alteration in migration of individual proteins in these gels by multiple methylation (10), the disappearance of the major methylated-protein species suggests that DmcA is a methylated protein. Since Northern blot analysis also indicated that the *dmcA* gene was not part of an operon structure, the mutation could directly affect only a single gene involved in chemotaxis. Therefore, the gene must play a crucial role in chemotaxis toward certain nutrients, which is consistent with its role as an MCP. Furthermore, these results taken together strongly support the hypothesis that the *dmcA* gene codes for an MCP in the oral spirochete. Recent results in our laboratory have also identified another ORF which contains a sequence homologous to the HCD in strain ATCC 35405 (2a) and may correspond to another specific MCP in these organisms. However, the precise number of MCPs present in *T. denticola* remains to be determined. It is of interest that the apparent size of the DmcA protein is similar to that of one of the proteins methylated following chemoattraction in the spirochete *Spirochaeta aurantia* (11). Likewise, an MCP gene from *Treponema pallidum* has recently been isolated (9).

Since the identification of chemoattractants or repellents for *T. denticola* and other spirochetes is limited (8), the isolation of an MCP gene in one spirochete now opens the way for identifying some of these signaling molecules. It will be of interest to identify the attractants and determine if their levels are elevated in the environment of inflamed human periodontal lesions. Such information might help to determine whether these organisms play a direct role in the development of such diseases. This approach is somewhat limited currently, since animal models for testing the virulence of *T. denticola* are still under development (7). Nevertheless, the utilization of mutants in in vitro model systems (3, 21) should help to identify the virulence factors of these organisms.

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