

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

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Immediately downstream from the previously isolated *Treponema denticola* ATCC 35405 *prtB* gene coding for a chymotrypsinlike protease activity, an open reading frame, ORF3, was identified which shared significant homology with the highly conserved domains (HCDs) of bacterial methyl-accepting chemotaxis proteins (MCPs). Nucleotide sequencing of this ORF revealed that the gene would code for a protein with a size of approximately 41 kDa. In addition, this sequence contained a domain which was virtually identical to the HCD of a recently characterized MCP, DmcA, of strain 35405. Therefore, this ORF was named *dmcB*. Northern blot analysis suggested that *dmcB* was part of an operon structure containing *prtB*. Insertional inactivation of *dmcB* utilizing an *ermF-ermAM* cassette resulted in a mutant with decreased chemoattraction toward nutrient supplements. In addition, the mutant displayed an altered pattern of methylated proteins under conditions of chemotaxis. Inactivation of the *dmcB* gene also attenuated the methylation of the DmcA protein. These results suggest that the *dmcB* gene codes for an MCP in *T. denticola* which may interact with other MCPs in these organisms.

Although a variety of anaerobic microorganisms have been associated with human periodontitis (10), spirochetes are among the most numerous organisms identified in disease sites (17). However, many of these organisms cannot be grown in the laboratory and have yet to be characterized. One of the cultivable spirochetes isolated from diseased tissue is the small spirochete *Treponema denticola* (6). The presence of these organisms in the oral cavity could be positively correlated with the severity of periodontitis (23). Therefore, these organisms may play a direct role in periodontal inflammation and may also serve as a model organism for characterizing the uncultivable oral spirochetes.

A number of potential virulence properties have been associated with *T. denticola*, including proteolytic activity, cytotoxicity, immune modulation, and the ability to invade tissue (7). Since the spirochetes are motile organisms and express flagella (5), it is likely that tissue invasion is dependent upon such expression. Likewise, *T. denticola* displays both positive (13) and negative (4) chemotaxis in vitro, and the former property may play a role in tissue invasion. Motile bacteria normally express *mcp* and *che* genes (25) in order to regulate chemotactic behavior. The methyl-accepting chemotaxis proteins (MCPs) interact with specific ligands, while the Che proteins relay the appropriate signals from the MCPs to the flagellar motor. Recently, an MCP gene, *dmcA*, was isolated from *T. denticola* and characterized (13). This protein was demonstrated to be required for chemotaxis toward nutrients. Moreover, the methylated protein profiles of *T. denticola* 35405 suggested the presence of other MCPs besides DmcA. The present communication describes the characterization of a second MCP in *T. denticola*, DmcB, with unique properties.

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MATERIALS AND METHODS

Bacterial strains and plasmids. *T. denticola* ATCC 35405 was maintained and grown in TYGVS broth medium (13) at 37°C in an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, Mich.) with an 85% N₂, 10% H₂, and 5% CO₂ atmosphere. *Escherichia coli* JM109 and MCP-deficient RP8611 (13) were utilized as host strains where indicated and were grown in Luria-Bertani broth (19). Plasmid pSA2 containing the *dmcB* gene (previously named open reading frame [ORF3]) was described earlier (1). *T. denticola* mutants were grown in TYGVS medium or on agar plates containing the same medium with erythromycin (Erm [40 µg/ml]).

Nucleotide sequencing. The nucleotide sequence of the *dmcB* gene was determined by a dideoxynucleotide sequencing strategy (20) with overlapping DNA fragments from the gene subcloned into pUC118, pUC119, or pBluescript II KS+ and KS- (Stratagene, La Jolla, Calif.) (1).

Construction of *T. denticola* mutants. A *dmcB* mutant of strain 35405 was isolated by the strategy recently developed in this laboratory for construction of *T. denticola* mutants (16). Briefly, an Erm^r cassette from plasmid pVA2198 (8) was introduced as a *PvuII* fragment into the *PvuII* site of the *dmcB* gene cloned into plasmid vector pKmOZ18 (21). The resulting plasmid was then linearized with *XhoI*, and the mixture was electroporated into strain 35405 as recently described (16). Erm^r transformants were isolated on TYGVS-Erm agar plates, and purified colonies were selected for further characterization.

A similar strategy was also utilized to construct a *prtB* mutant (15a).

Expression of the *dmcB* gene in *E. coli*. The intact *dmcB* gene was isolated by PCR with two primers (5'-GGG GAT CCA AAA ACG GTA GAC GAT TTA-3', including the sequence immediately upstream from the start codon and containing a *BamHI* site, as well as 5'-GGG AAT TCT CGT TCA TAC GTC GTT AT-3', with the sequence immediately upstream of the termination codon, including an *EcoRI* site) and with plasmid pSA2 as a template under conditions recently described (13). The PCR fragment was then linearized with *BamHI* and *EcoRI* and ligated into similarly cleaved pUC118. This produced an in-frame fusion between the *lacZ'* and *dmcB* genes under the control of the *lac* promoter.

Southern blot analysis. Chromosomal DNA was digested with various restriction enzymes, transferred to nylon membranes, hybridized with the indicated probes, and analyzed as previously described (13) with the enhanced chemiluminescence (ECL) direct labeling and detection systems (Amersham International, Plc., Amersham, Buckinghamshire, United Kingdom).

Western blot analysis. For detection of cross-reactive *T. denticola* proteins with anti-MCP sera, anti-Trg serum (18) (kindly provided by R. Hazelbauer, Washington State University, Pullman) was utilized. This serum recognizes proteins from a broad spectrum of bacteria, including 20 different species. *T. denticola* or *E. coli* cell extracts were prepared, run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, transferred to Immobilon polyvinylidene difluoride membranes, and analyzed as previously described (13). Equal amounts of sample (micrograms of protein) were loaded in each lane, and the blots were developed with a 1:1,000 dilution of anti-Trg serum.

Northern blot analysis. RNA was extracted from *T. denticola* cells by using Trizol LS as described by the supplier (Gibco BRL, Gaithersburg, Md.). Approximately 70 µg of RNA was loaded onto formaldehyde-agarose gels and analyzed as previously described (13) by the ECL system with the indicated probes.

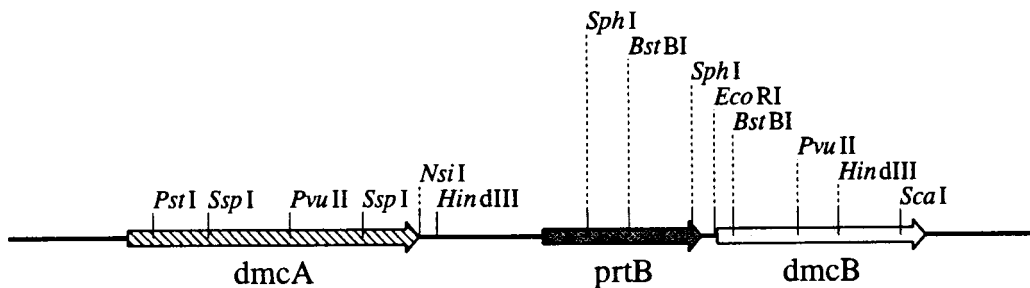


FIG. 1. Orientation of the *dmca*, *prtB*, and *dmcB* genes on the *T. denticola* 35405 chromosome. Bar, 1.0 kb.

RT-PCR. RNA from *T. denticola* was treated with RNase-free DNase (0.2 U of DNase/μg of RNA) in buffer (2) for 2 h at 37°C and extracted with Trizol LS reagent, and the RNA was precipitated with isopropanol. Three to 5 μg of RNA was incubated with random hexamers in PCR buffer-MgCl₂-deoxynucleoside triphosphates for 5 min at 42°C. SuperScriptII RT reverse transcriptase (Gibco) was then added, and the mixture was incubated at 42°C for 1 h. The reaction was terminated by incubation at 95°C for 5 min, and the mixtures were treated with RNase H at 37°C for 30 min. PCR was then carried out with the indicated primers (denaturation at 94°C for 4 min, followed by 40 cycles of denaturation at 94°C for 1 min and annealing at 64°C for 1 min and 72°C for 1 min, followed by extension at 72°C for 10 min. The PCR products (10 μl) were loaded onto agarose gels and analyzed as described earlier (12).

Methylation analysis. Methylation of MCPs in *T. denticola* was carried out as previously described (13). Cells stimulated with rabbit serum and nutrients were labeled with [³H]methionine, the proteins were separated by SDS-PAGE, and the labeled proteins were detected following fluorography.

Chemotaxis assays. Chemotaxis toward nutrient supplements (serum plus nutrients) was carried out with the two-phase system as previously described (16). Briefly, the top agarose layers containing TYGVS medium and *T. denticola* cells were overlaid onto the same medium containing serum and glucose. The tubes were incubated at 37°C anaerobically for 7 to 10 days, and migration into the bottom layers was monitored visually.

Nucleotide sequence accession number. The nucleotide sequence of the *dmcB* gene has been deposited in the GenBank database under accession no. U84257.

RESULTS

Characterization of ORF3. Previously, we isolated a gene, *prtB*, coding for a chymotrypsinlike protease activity (1) from *T. denticola* 35405 on plasmid pSA2. Upstream from this gene, another gene, *dmcA* (Fig. 1), expressing an MCP was identified and characterized (13). Partial sequencing downstream from the *prtB* gene revealed the presence of another putative gene, ORF3, which has now been completely sequenced (Fig. 2). The gene would code for a protein with an approximate molecular size of 41 kDa. Immediately upstream from the potential start codon, a sequence, AGG, that may act as a ribosome binding site was identified. No sequences corresponding to the consensus promoter sequence of *E. coli* were identified upstream of the start codon. A Kyte and Doolittle hydrophathy plot revealed one significant hydrophobic domain encompassing the N-terminal region of the protein (data not shown). This is in contrast to most bacterial MCPs, which contain two distinct hydrophobic regions (25).

A comparison of the amino acid sequences of ORF3 with sequences in the NBRF database revealed the highest homology with a variety of bacterial MCPs. This homology was confined to the C-terminal chemotaxis signaling regions of these proteins, as exemplified by the *E. coli* Tap protein (15) (Fig. 3). In addition, this region was highly homologous to a similar domain from the recently characterized *T. denticola* Dmca MCP. Such similarity suggested the possibility that ORF3 might also code for a protein involved in the chemotactic

response of *T. denticola*. ORF3 also displayed extensive similarity (66% identity at the amino acid level over 348 amino acids) to an uncharacterized ORF (TIGR/TP5582) from the recently released *Treponema pallidum* genome sequence.

Expression of ORF3 in *E. coli*. In order to further characterize the ORF3 product, a DNA fragment containing the

1	ATTAAGCACC	<u>HincII, EcoRI</u>	CGGTTAACGAATTCATAAAAA	CGGTAGACGATTTAAGGATGAGTATAAGA	60
				M S I R	4
61	ATTTTAGGCAT		TATCATTTTGGATTATTACACTTGC	CGGTATATATGCCGTCGCACATTAT	120
	5 I L G I I I L I I T L A V I Y A V A H Y				24
121	ATGCTCCATCC	<u>BulI</u>	TATTCGAAAAACGGTTTCGGCGTT	AAAAAGATATTGCACAGGGAAAAGGA	180
	25 M L H P I R K T V S A L K D I A Q G K G				44
181	GACTTAACGT		TCGCCCTCCCATTAACGGAAATG	TGAATCAACCGATTGTCGCAATAT	240
	45 D L T V R L P I N G N D E I T D L S E Y				64
241	TTCAATCAA		CAAAATGAAAAATAGGCGCTG	CAATCAAAATGTCGGTAAAAATTCGGTT	300
	65 F N Q T I E K I G A A I K N V G K N S V				64
301	TCAATGGA		GCCATGAGGAGGAACCTTGCTT	CCAATGACTCAAACGGCGAGTGTATA	360
	85 S M E A A I G E E L A S N M T Q T A S A I				104
361	AATGAATC		AGTGCACAAATATTGAAGGGTT	AAAAACCAACCCCTTACGCAGGCTGCAAGC	420
	105 N E I S A N I E G V K N Q T L T Q A A S				124
421	GTAACAGAA	<u>PvuII</u>	CTGCAGTACAAATGAACAAAT	TGACCCACTATAAACAGCTGAATAAC	480
	125 V T E T A A T I E Q I V R T I K Q L N N				144
481	AGTAGAAA		ATCAGGCAAGCGGTACAGCT	TCAATCGAAGAAATGGTT	540
	145 S I E N Q A A S V A R S T A S I E E M V				164
541	GCAACATCG		ATCCATCTCAACACTTGA	AAAAACCTATGATGTCGTA	600
	165 A N I A S I T Q T L E K T Y D V V K N L				184
601	GCATCGGCA		CTGAAAGCGGAAAGAACAG	TATTAACCTCAAACAGCGTAAACAGAAA	660
	185 A S A T E D G K E T I I T S N S V T Q K				204
661	ATTGCAAG	<u>HindIII</u>	AGTCCGGCTCGCTCATGGA	AGGTTCAAGCGTTATTCAGCATATGCAAGC	720
	205 I A E E S G S L M E A S S V I Q H I A S				224
721	CAACCAAT		TGGTTCGAATGAATGCG	CTATTGAAGCTGCCCATCGACGGCAAGCAGGC	780
	225 Q T N L L A M N A A I E A A H A G E A G				244
781	AAAGSTTT		GCTGCGTCGCGATGAAAT	CAGAAAGCTCGCTGAAGACTCGGCACTCAA	840
	245 K G F A V V A D E I R K L A I D S A T Q				264
841	GGCAAGCA		ATAACGCTACCGCTTAA	ACCCCTTCAAGCGAAATAGATCTCTTCGGTT	900
	265 G K T I T S T L K A A L S S E I E T S L S V				284
901	TCTTCTAAA		CCGTCGAAGACAAATTC	CAGCATTTTAAATTTATCCGAACAAGTAAA	960
	285 S S K T V E D K F S T I F N L S E Q V K				304
961	TCAATGAG	<u>ScaI</u>	TGACCCCTTACCGAAGCG	ATGCAAGCAGGCAAGCGGCAAGCAAGTA	1020
	305 S M S D R L T E A M H E D G E N S K E V				324
1021	CTCGSAGCA		TAAAAATATCAATACGGT	AACGGTGAAGTGAAGTGAAGTTCGCGGTTCCGAGAG	1080
	325 L G A I K N I N T V T V E V Q A G S E E				344
1081	ATGCTGAA		GGCGGTGAAGGAGTGC	TGAAGAAATGCTGAAACTTGATAATTAACCCGT	1140
	345 M L K G G E G V A E M A L K L D N L T R				364
1141	ATGATAAC		GCAGATGACGAGATGG	CAGCGTCCGGTTCAGAT	1186
	365 M I T T V *				369

FIG. 2. Nucleotide and deduced amino acid sequences of the *dmcB* gene. Major restriction enzyme sites are also indicated. *, TGA stop codon.

<i>E. coli</i> Tap (208)	..WTRKMIIVQPLAIIIGSHFDSIAA-----GNLARP!
<i>T. d</i> <i>dmcB</i> (1)	..MSIRILGIILIIITLAVIYAVAHYMLHPIRKTVSALKDIAQGGKDLIVRL
<i>T. d</i> <i>dmcA</i> (369)	..IKT-----
<i>E. coli</i> Tap (238)	..AVYGRNEITAIFASLKTMQQALRGTVSIVRKGSDMHIQ-----
<i>T. d</i> <i>dmcB</i> (51)	..PINGNDEITDLSEYFNQTIIEKIGAAIKNVGKNSVSMEDIAEELASNMQT
<i>T. d</i> <i>dmcA</i> (372)	..-VYG-----
<i>E. coli</i> Tap (277)	..---IAEIVAGNNDLSSRTEQDAASLAQTAASMEQLTATVYQQ-NAQNRQA
<i>T. d</i> <i>dmcB</i> (101)	..ASAINEISANIEGYKQNTLTQAASVYETETAATIEQIVRTIKQLNNSIENGA
<i>T. d</i> <i>dmcA</i> (375)	..-----QTKV-GKDGARTA
<i>E. coli</i> Tap (323)	..SELA-----KNAATTAQGGGVQVSTMT
<i>T. d</i> <i>dmcB</i> (151)	..ASVARTASIEEMVANIAITQTLEKTYBYVKNLASATEQKETIIISNS
<i>T. d</i> <i>dmcA</i> (387)	..NEIV-----K-----
<i>E. coli</i> Tap (346)	..TMQEIATSSQKIGDIISYIDGIAFDTNILALNAAVEAARAGEQGRGFAYV
<i>T. d</i> <i>dmcB</i> (201)	..VTGKIAEESGSLMEASSVICHIASQTNLLAMNAAIEAAHAGEAGKGFAYV
<i>T. d</i> <i>dmcA</i> (391)	..---QIAEKSAASLLEASQIIQNIASQTNLLAMNAAIEAAHAGEGSKGFAYV
(Chemotaxis signaling)	
<i>E. coli</i> Tap (396)	..AGEVNRNLASRSADAAKEIKGLIEESVNRVQGGSKLYNNAATMIDIVSSV
<i>T. d</i> <i>dmcB</i> (251)	..ADEIRKLAEDSATQGKTI-----TSTLTKLSSEIESLSVSSK
<i>T. d</i> <i>dmcA</i> (438)	..ADEIRKLAEGSNLQKQIAAVIKE-----

FIG. 3. Comparison of the amino acid sequences of the DmcB protein with those of the *E. coli* Tap and *T. denticola* (*T. d*) DmcA proteins. Conserved amino acid sequences are denoted by the colons. The putative chemotaxis signaling HCD is underlined. The numbers refer to the amino acid positions of each protein.

entire ORF was cloned into the expression vector pUC118. Following induction of ORF3 expression in the presence of isopropyl- β -D-thiogalactopyranoside (IPTG), a protein band of approximately 40 kDa was detected following Western blot analysis with antibody against the *E. coli* Trg protein (Fig. 4), but this band was not detected following protein staining relative to the strain harboring only the vector. A similar-size band was also detected with this antibody from an analysis of proteins expressed from plasmid pSA2 (data not shown). These results suggested that ORF3 codes for a protein with homology to MCPs, such as Trg. Therefore, the ORF was tentatively named *dmcB* (for *denticola* MCP B).

Transcription of *dmcB* in *T. denticola*. Since an examination of the nucleotide sequence of *dmcB* and the previously char-

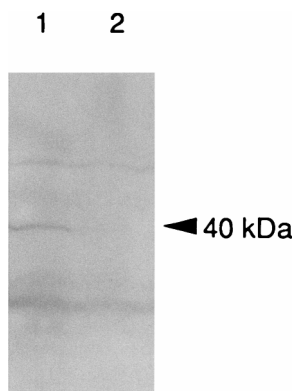


FIG. 4. Western blot analysis of the expression of the DmcB protein in *E. coli*. Lanes: 1, *E. coli* RP8611 containing pUC118/*dmcB*; 2, RP8611 containing plasmid vector pUC118. The arrows indicate the 40-kDa protein detected with anti-Trg antibody.

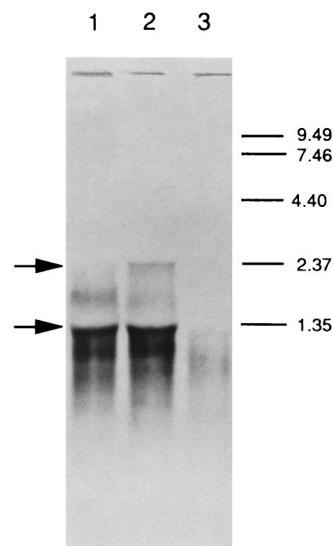


FIG. 5. Northern blot analysis of *T. denticola* RNA with the *dmcB* probe. Lanes: 1, *dmcB* mutant strain HL503; 2, parental strain 35405; 3, *prtB* mutant strain HL502. Conditions for the analysis are indicated in the text. The arrows indicate the major mRNA species. Molecular size markers (kilobases) are depicted in the right margin.

acterized *prtB* gene did not indicate the presence of a potential promoter sequence upstream of the former gene, it was of interest to determine if the two genes were part of the same polycistronic mRNA. Northern blot analysis (Fig. 5) revealed that strain 35405 expressed a message of approximately 2.3 kb according to a *dmcB* probe. A similar-size species near 2.0 kb was also indicated for the mRNA detected with the *prtB* probe (1). An additional positive band at approximately 1.3 kb was also apparent, but its identity is unknown, and it could represent binding of the mRNA to either rRNA or the *prtB* transcript. Furthermore, RT-PCR analysis using one primer from the *prtB* gene and another from the *dmcB* gene revealed a positive band consistent with cotranscription of both genes (data not shown).

Construction of a *dmcB* mutant. In order to determine the role of the *dmcB* gene in the physiology of *T. denticola*, a recently developed gene inactivation system for these spirochetes (16) was utilized to construct a monospecific *dmcB* mutant (Fig. 6). An *Erm*^r cassette was introduced into the internal *Pvu*II site of the gene, and the linearized plasmid fragment was electroporated into strain 35405. *Erm*^r colonies were isolated, and Southern blot analysis (Fig. 7) confirmed the replacement of the intact *dmcB* gene with the inactivated copy. Positive bands in one of the selected transformants, HL503, demonstrated the predicted increased sizes when chromosomal DNA was cleaved with several restriction enzymes and examined with a *dmcB* probe. Likewise, no positive hybridizing bands were detected with the *Erm* probe in strain 35405, but they were present in mutant HL503. These results confirmed the insertion of the *Erm* cassette into the *dmcB* gene in the mutant. In addition, Northern blot analysis of the mutant revealed the disappearance of the 2.3-kb mRNA, but not the 1.3-kb mRNA, following inactivation of the *dmcB* gene (Fig. 5). Western blot analyses of extracts of the mutants were inconclusive due to the weak cross-reactivity of anti-Trg antibody with the relatively low levels of DmcA and DmcB present in *T. denticola*.

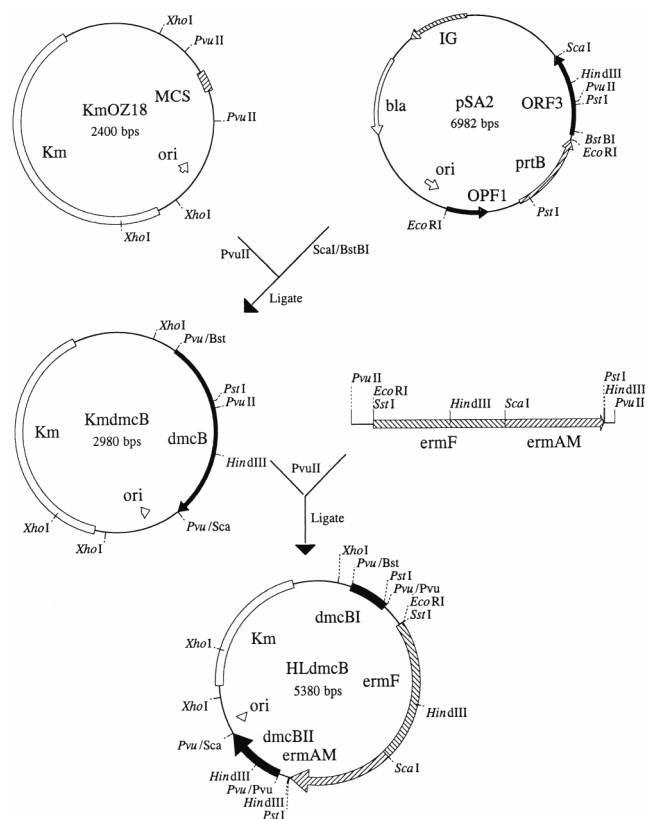


FIG. 6. Strategy for constructing the *dmcB* mutant. The Erm cassette from pVA2198 was introduced into the *PvuII* site of the *dmcB* gene (also indicated as ORF3) contained on plasmid pSA2. The resultant plasmid, pHLdmcB, was linearized following *XhoI* cleavage and utilized to transform strain 35405.

Properties of the *dmcB* mutant. Since the apparent homology of the DmcB protein with MCPs suggested that the protein may play a role in chemotaxis, it was of interest to examine the methylation profiles of mutant HL503. Following stimulation of the parental organism with serum plus nutrients, a number of methylated protein bands were apparent (Fig. 8). A series of bands around 35 to 43 kDa likely represent different methylated forms of DmcB, while the strong band at approximately 65 kDa is the DmcA methylated protein (13). In both the *prtB* and *dmcB* mutants, the strong bands in the 40-kDa region were absent, as would be predicted for a role of the DmcB protein in methylation. Interestingly, the DmcA protein was also undermethylated in the mutants. Therefore, inactivation of the *dmcB* gene attenuated methylation not only of DmcB but also of DmcA. The identities of the other methylated bands are currently unknown. *T. denticola* may express other MCPs in addition to DmcA and DmcB, as observed in other bacteria (25).

In order to determine if the *dmcB* gene product plays a role in chemotaxis, mutant HL503 was compared to the parental organism in the agarose-layered chemotaxis system developed in our laboratory (16). Visible migration of parental strain 35405 into the agarose layer containing serum plus nutrients was readily observed (Fig. 9). However, movement of HL503 into this layer was inhibited. These results directly demonstrate that the *dmcB* gene plays a role in chemotaxis toward nutrients. However, mutant HL503 was not altered in repulsion from agar (16).

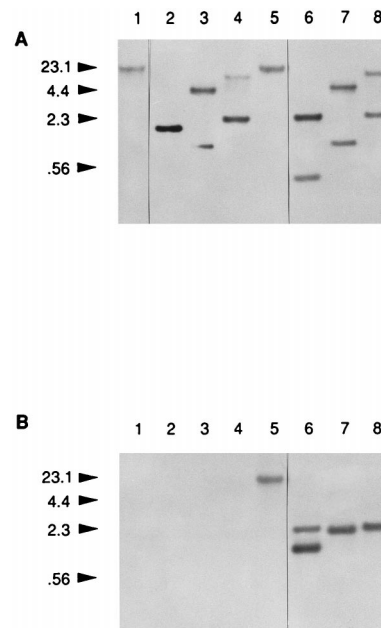


FIG. 7. Southern blot analysis of the *dmcB* mutant, HL503. Lanes: 1 to 4, parental 35405; 5 to 8, mutant HL503; 1 and 5, uncut chromosomal DNA; 2 and 6, *HindIII* cleaved; 3 and 7, *PstI* cut; 4 and 8, *PvuII* cleaved. The probe for panel A was the 0.53-kb *BstBI-HindIII* fragment of the *dmcB* gene, while that for panel B was the 2.1-kb *SsrI-PstI* fragment from the Erm cassette. The numbers in the left margin indicate the molecular size markers (kilobases).

DISCUSSION

The demonstration that oral spirochetes can penetrate oral tissue in inflamed sites (17) suggested that these organisms may contain the chemotaxis genes present in other motile bacteria (25). Previously, it was demonstrated that, at least for one oral spirochete, *T. denticola*, chemotaxis away from inhibitory agar media could be demonstrated (4). Furthermore, with an adaptation of the latter in vitro assay system, chemotaxis of strain 35405 toward nutrients was confirmed (13). Therefore, it was reasonable to predict that *mcp* and *che* genes would be present in these organisms.

The recent isolation and characterization of a chemotaxis gene, *dmcA*, from strain 35405 suggested the presence of a

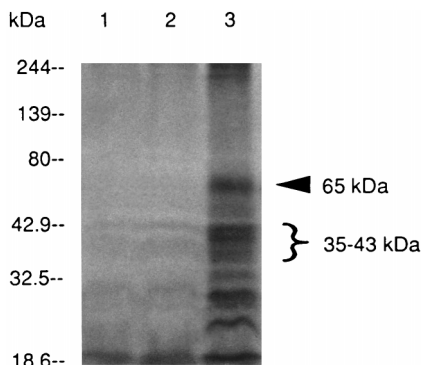


FIG. 8. Protein methylation patterns of the parental as well as the *dmcB* and *prtB* mutants. The cells were prepared and stimulated as described in the text. Lanes: 1, *dmcB* mutant HL503; 2, *prtB* mutant HL502; 3, parental strain 35405. The numbers on the left indicate the molecular mass markers. The designations on the right indicate the major proteins which appear to be undermethylated in the mutant strains.

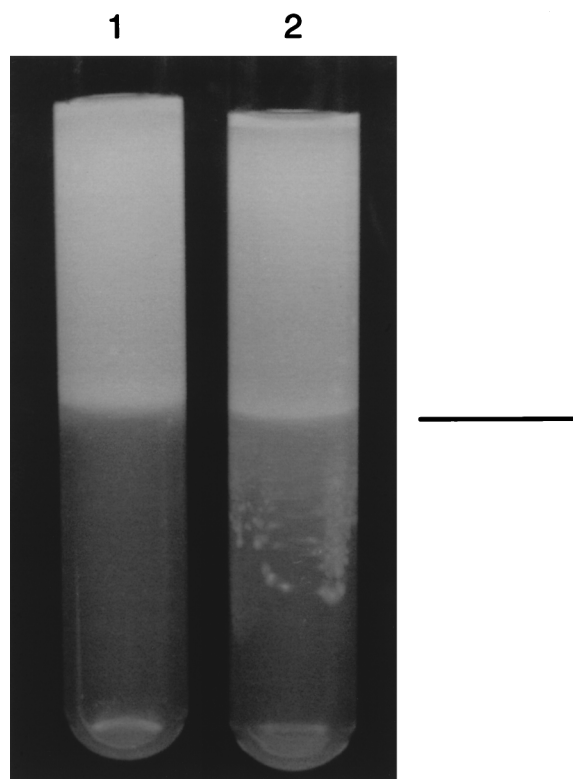


FIG. 9. Chemotactic behavior of the *dmcB* mutant. Following inoculation of the top layer (TYGVS-agarose) with the bacteria, the migration of the cells into the bottom layer (TYGVS-agarose containing serum and nutrients) was monitored. Tube 1, *dmcB* mutant strain HL503; tube 2, parental strain 35405. The line in the right margin represents the boundary between the top and bottom layers.

chemotaxis system in *T. denticola* (13). The same gene was also recently isolated from a strain 35405 lambda phage clone bank (9). Inactivation of the *dmcA* gene indicated that this gene was required for chemotaxis toward nutrients, but not for negative chemotaxis relative to inhibitory agar. The present investigation documents the presence of a second chemotaxis gene, *dmcB*, on the chromosome of strain 35405. This gene is not present within the same operon as *dmcA*, but it is cotranscribed with the *prtB* gene, which codes for a chymotrypsinlike protease. However, the latter gene is distinct from the *prtP* gene, which appears to code for the major cell surface chymotrypsinlike protease in *T. denticola* (11). Interestingly, the *prtB* gene is flanked by both the *dmcA* and *dmcB* genes. Since genes of similar function are normally clustered together, this arrangement suggests that the *prtB* gene could play a role in chemotaxis, but this has yet to be established.

One putative domain of the DmcB protein shares extensive homology with the highly conserved domains (HCDs) of many bacterial MCPs. In addition, the HCD of DmcB is virtually identical in amino acid sequence to the same domain in DmcA. The molecular mass of DmcB, approximately 41 kDa, is significantly smaller than that of DmcA, with a revised molecular mass of 70 kDa, as well as those of most other bacterial MCPs (25). However, lower molecular masses have also been suggested for MCPs in other spirochetes (18). It is of interest that a homologous protein of this approximate molecular size has also been detected on the *T. pallidum* genome. These smaller MCPs may therefore be characteristic of members of the *Treponema* and other spirochete genera.

Unlike most MCPs, which exhibit two characteristic hydro-

phobic domains which are believed to span the cytoplasmic membrane (25), the DmcB protein contains only a single hydrophobic domain capable of such orientation. Therefore, it will be of interest to determine how the latter protein is localized in *T. denticola* relative to the cytoplasmic membrane. Recently, it has been suggested that another MCP, McpA from *Rhodobacter sphaeroides*, also contains only a single large hydrophobic domain (26).

Another unusual feature of the DmcB protein is that inactivation of the corresponding gene also affected the methylation of the DmcA protein. However, the mechanism of such interactions remains to be determined. In addition, these results could indicate that transmethylation between the two proteins may occur in *T. denticola*. Such interaction between distinct MCPs has also been suggested to occur in *Bacillus subtilis* (3). The alternative explanation, that inactivation of the *dmcB* gene somehow affects the expression of the *dmcA* gene, is suggested by the weak DmcA protein bands observed on Western blots of the *dmcB* mutant (data not shown). Therefore, it is not clear if the chemotaxis defects displayed by the *dmcB* mutant resulted directly from an alteration of this gene product or were the result of methylation attenuation of the DmcA protein. Interestingly, inactivation of the *dmcA* gene does not appear to affect the methylation of the DmcB proteins (13). These results suggest that DmcB may play a unique role as an intermediate in the methylation and regulation of the expression of DmcA or other MCPs. Confirmation of this hypothesis will require additional approaches.

The demonstration of the presence of at least two MCPs in *T. denticola*, as well as recent reports of the presence of *che* genes in these organisms (22, 24), suggests that these organisms may contain the same basic complement of chemotaxis proteins which are present in other motile bacteria. The recent development of a gene transfer system for these organisms (16) now makes it possible to determine the role of these genes in chemotaxis as well as in the virulence of these periodontopathic bacteria by utilizing animal model systems (14).

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REFERENCES

1. Arakawa, S., and H. K. Kuramitsu. 1994. Cloning and sequence analysis of a chymotrypsinlike protease from *Treponema denticola*. *Infect. Immun.* **62**:3424-3433.
2. Bauer, P., A. Rolf, V. Regitz-Zagrosek, A. Hildebrandt, and E. Fleck. 1997. Use of manganese in RT-PCR eliminates PCR artifacts resulting from DNaseI digestion. *BioTechniques* **22**:1128-1132.
3. Bedale, W. A., D. O. Nettleton, C. S. Sopata, M. S. Thielke, and G. W. Ordal. 1988. Evidence for methyl group transfer between the methyl-accepting chemotaxis proteins in *Bacillus subtilis*. *J. Bacteriol.* **170**:223-227.
4. Chan, E. C. S., Y.-S. Qui, R. Siboo, and P. Noble. 1995. Evidence for two distinct locomotor phenotypes of *Treponema denticola*. ATCC 35405. *Oral Microbiol. Immunol.* **10**:122-124.
5. Charon, N. W., E. P. Greenberg, M. B. H. Koopman, and R. J. Limberger. 1992. Spirochete chemotaxis, motility, and the structure of the spirochetal periplasmic flagella. *Res. Microbiol.* **143**:597-603.
6. Cheng, S.-L., R. Siboo, T. Chin Quee, J. L. Johnson, W. R. Mayberry, and E. C. S. Chan. 1985. Comparative study of six random oral spirochete isolates. Serological heterogeneity of *Treponema denticola*. *J. Periodontol. Res.* **18**:602-612.
7. Fenno, J. C., and B. C. McBride. 1998. Virulence factors of oral treponemes. *Anaerobe* **4**:1-17.
8. Fletcher, H. M., H. A. Schenkein, R. M. Morgan, K. A. Bailey, C. R. Berry, and F. L. Macrina. 1995. Virulence of a *Porphyromonas gingivalis* W83 mutant defective in the *prtH* gene. *Infect. Immun.* **63**:1521-1528.
9. Greene, S. R., and L. V. Stamm. 1997. Identification, sequence, and expression of *Treponema denticola* *mcpA*, a putative chemoreceptor gene. *FEMS Microbiol. Lett.* **157**:245-249.

10. Haffajee, A. D., and S. S. Socransky. 1994. Microbial etiological agents of destructive periodontal diseases. *Periodontol.* 2000 **5**:78–111.
11. Ishihara, K., T. Miura, H. K. Kuramitsu, and K. Okuda. 1996. Characterization of the *Treponema denticola* *prtP* gene encoding a prolyl-phenylalanine-specific protease (dentilisin). *Infect. Immun.* **64**:5178–5186.
12. Karunakaran, T., T. Madden, and H. Kuramitsu. 1997. Isolation and characterization of a hemin-regulated gene, *hemR*, from *Porphyromonas gingivalis*. *J. Bacteriol.* **179**:1898–1908.
13. Kataoka, M., H. Li, S. Arakawa, and H. Kuramitsu. 1997. Characterization of a methyl-accepting chemotaxis protein gene, *dmcA*, from the oral spirochete *Treponema denticola*. *Infect. Immun.* **65**:4011–4016.
14. Kesavalu, L., S. G. Walker, S. C. Holt, R. R. Crawley, and J. L. Ebersole. 1997. Virulence characteristics of oral treponemes in a murine model. *Infect. Immun.* **65**:5096–5102.
15. Krikos, A., N. Mutoh, A. Boyd, and M. I. Simon. 1983. Sensory transducers of *Escherichia coli* are composed of discrete structural and functional domains. *Cell* **33**:615–622.
- 15a. Li, H., and H. Kuramitsu. Unpublished data.
16. Li, H., J. Ruby, N. Charon, and H. Kuramitsu. 1996. Gene inactivation in the oral spirochete *Treponema denticola*: construction of an *flgE* mutant. *J. Bacteriol.* **178**:3664–3667.
17. Loesche, W. J. 1988. The role of spirochetes in periodontal disease. *Adv. Dent. Res.* **2**:275–283.
18. Morgan, D. G., J. W. Baumgartner, and G. L. Hazelbauer. 1993. Proteins antigenically related to methyl-accepting chemotaxis proteins of *Escherichia coli* detected in a wide range of bacterial species. *J. Bacteriol.* **175**:133–140.
19. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
21. Sato, Y., Y. Yamamoto, R. Suzuki, H. Kizaki, and H. K. Kuramitsu. 1991. Construction of *scrA::lacZ* gene fusions to investigate regulation of the sucrose PTS of *Streptococcus mutans*. *FEMS Microbiol. Lett.* **79**:339–346.
22. Shi, W. Personal communication.
23. Simonson, L. G., C. H. Goodman, J. J. Bial, and H. E. Morton. 1988. Quantitative relationship of *Treponema denticola* to severity of periodontal disease. *Infect. Immun.* **56**:726–728.
24. Stamm, L. V. Personal communication.
25. 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. Chem.* **20**:109–136.
26. Ward, M. J., D. M. Harrison, M. J. Ebner, and J. P. Armitage. 1995. Identification of a methyl-accepting chemotaxis protein in *Rhodobacter sphaeroides*. *Mol. Microbiol.* **18**:115–121.

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