INFECTION AND IMMUNITY, June 1991, p. 1941–1947
0019-9567/91/061941-07$02.00/0

Vol. 59, No. 6

Molecular Cloning and Sequence Analysis of Antigen Gene *tdpA* of *Treponema denticola*

MANABU MIYAMOTO,[1] SUMIHARE NOJI,[2] SUSUMU KOKEGUCHI,[3] KEIJIRO KATO,[3] HIDEMI KURIHARA,[1] YOJI MURAYAMA,[1] AND SHIGEHIKO TANIGUCHI[2]*

Department of Periodontology and Endodontology,[1] Department of Biochemistry,[2] and Department of Oral Microbiology,[3] Okayama University Dental School, Shikata-cho, Okayama City 700, Japan

Received 27 August 1990/Accepted 13 March 1991

We isolated and characterized an immunogenic protein of an oral spirochete, *Treponema denticola* Johnson. A genomic DNA library constructed with bacteriophage lambda EMBL3 as a vector was immunologically screened with a rabbit antiserum against the whole cells. Using Western immunoblot analysis, we found a particular clone encoding an antigen with a molecular weight of 53,000; we designated the antigen as *T. denticola* protein A (TdpA). Complete sequence determination revealed an open reading frame of 1,419 bp and a signal peptide sequence that was homologous to that of bacterial lipoprotein. Southern hybridization analysis revealed that the *tdpA* gene is highly conserved in six tested strains of *T. denticola* species. Furthermore, we found that sera from some periodontitis patients contained antibody against the TdpA protein, although the reactivities of the antibodies varied among individuals.

Oral spirochetes are the dominant bacteria in the subgingival plaque of patients with periodontal disease. An absolute increase in their population and in proportion to other oral bacteria is generally observed in periodontal pockets with concomitant progression of the disease (for a review, see reference 16). However, their role as etiologic agents of periodontal disease has not yet been fully elucidated. Isolation of antigens specific to oral spirochetes is important for evaluating their role in either the initiation or progression of periodontal disease. A sodium deoxycholate-ethanol-extractable antigen (10) and a factor inhibitory to fibroblast proliferation (2) have been reported to be antigens of oral spirochetes; however, neither was purified enough to define its virulence and immunogenicity. To circumvent the problems through isolation of satisfactorily characterized antigens of these fastidious organisms, we applied recombinant DNA technology. In this paper, we report the molecular cloning and sequence analysis of an antigen gene of *Treponema denticola*. From the deduced amino acid sequence, some characteristics of the antigen protein are predicted.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in this study are listed in Table 1. *T. denticola* Johnson was chosen for all cloning procedures and antiserum preparations. The spirochetes were cultured in our modified NOS medium (15) containing 12.5 g of brain heart infusion (Difco Laboratories, Detroit, Mich.), 10 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 2.5 g of yeast extract, 0.5 g of sodium thioglycolate, 1 g of L-cysteine, 0.25 g of L-asparagine, 0.25 g of sodium pyruvate, 0.4 g of nicotinamide, 2 g of glucose, 6 mg of cocarboxylase, and 2 g of sodium bicarbonate per liter and 2% (vol/vol) horse serum. Volatile fatty acids were supplied to this medium for culturing *Treponema socranskii*, *Treponema* sp. strain G7201, and *Treponema pallidum*. The cultures were incubated at 37°C for 1 week in an anaerobic chamber containing 80% N_2, 10% H_2, and 10% CO_2. The bacterial cells were harvested by centrifugation at 10,000 × *g* for 20 min at 4°C and then washed twice with phosphate-buffered saline (PBS) at pH 7.2. *Escherichia coli* LE392 and Q359 were grown in LB medium. *E. coli* XL1-Blue was maintained in LB medium containing tetracycline (12.5 μg/ml). When necessary, the growth media were supplemented with ampicillin (50 μg/ml), isopropyl-β-D-thiogalactopyranoside (5 mM), and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (40 μg/ml).

Antisera. An antiserum against *T. denticola* was raised by the subcutaneous immunization of a rabbit with whole cells of *T. denticola* (suspended in PBS) with Freund complete adjuvant once a week for 5 weeks; then a single intravenous booster was administered. The serum of the rabbit was obtained 3 days after the last immunization. For immunoblot analyses, the rabbit antiserum was extensively absorbed with intact *E. coli* LE392 cells and further with the sonic extracts to minimize the amounts of antibodies against *E. coli* proteins. Human sera were obtained from patients with periodontitis at the Okayama University Dental Hospital as well as from periodontally healthy subjects. The sera tested were randomly selected among the patients showing high titers of serum antibody against *T. denticola*. The immunoglobulin G antibody titers to *T. denticola* were determined by the method of Murayama et al. (20).

DNA extraction. Chromosomal DNA of *T. denticola* was prepared by the method of Van Embden et al. (31) with some modifications as follows. The bacterial cells (1.0 g [wet weight]) were suspended in 10 ml of 20% (wt/vol) sucrose–50 mM EDTA–50 mM Tris-HCl (pH 7.5). After the addition of DNase-free RNase (50 μg/ml) and proteinase K (0.3 mg/ml), the cells were lysed by further addition of sodium dodecyl sulfate (SDS) to 0.5% (wt/vol) and subsequent incubation for 60 min at 37°C. The DNA was extracted with phenol, phenol-chloroform, and finally chloroform. The recovered DNA was precipitated with ethanol and dissolved in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA.

Construction of *T. denticola* genomic library. The chromosomal DNA was partially digested with *Sau*3A and electrophoresed with low-melting-point agarose. The gel segments

* Corresponding author.

TABLE 1. Bacterial strains

Species and strain	Source and reference
Treponema denticola	
Johnson	T. Watanabe[a]
ATCC 35405	ATCC[b]
ATCC 35404	ATCC
ATCC 33520	ATCC
S-2	Clinical isolate, T. Eguchi[c]
OKA-3	Clinical isolate, T. Eguchi
Treponema species	
E-21	Clinical isolate (27), T. Watanabe
E-30	Clinical isolate (12), T. Watanabe
Y-181	Clinical isolate (1), T. Watanabe
G7201	Clinical isolate (28), T. Umemoto[d]
Treponema socranskii subsp. *buccale* ATCC 35534	ATCC
Treponema socranskii subsp. *socranskii* ATCC 35536	ATCC
Treponema pallidum ATCC 27087	ATCC
Porphyromonas gingivalis 381	S. S. Socransky[e]
Actinobacillus actinomycetemcomitans ATCC 29523	ATCC
Wolinella recta ATCC 33238	ATCC
Escherichia coli K-12	
LE392	21
Q359	11
XL1-Blue	3

[a] Department of Bacteriology, Nihon Dental University, Tokyo, Japan.
[b] American Type Culture Collection.
[c] Oral Care Laboratory, Sunstar Inc., Osaka, Japan.
[d] Department of Oral Microbiology, School of Dentistry, Asahi University, Gifu, Japan.
[e] Forsyth Dental Center, Boston, Mass.

containing the DNA fragments of 10 to 20 kb were excised and pooled. The DNA fragments were recovered from the gel by the method of Maniatis et al. (17). The size-fractionated DNA fragments were ligated to arms of bacteriophage EMBL3 *Bam*HI (Amersham, Buckinghamshire, United Kingdom). The ligated DNAs were packaged into phages, and then the recombinant phages were transduced into *E. coli* LE392. The total number of plaques obtained from the entire ligation mixture was 4×10^5.

Screening of *T. denticola* genomic library. Recombinant phages were plated with *E. coli* LE392; approximately 1,000 plaques per plate were produced. The plates were then cooled to 4°C and overlaid with nitrocellulose filters. After removal from the plates, the filters were blocked with 5% (wt/vol) skim milk in TBS (20 mM Tris-HCl [pH 7.5]–500 mM NaCl). They were then incubated for 2 h at room temperature in TBS containing the rabbit antiserum at a final dilution of 1:200 and 5% skim milk. After filters were washed with TBS containing 0.05% Tween 20, they were incubated for 1 h at room temperature in TBS containing horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G antibodies (1:500 dilution) and 5% skim milk and then washed again with TBS containing 0.05% Tween 20. The immunoreactive proteins were detected by immersing the filters in a substrate solution containing 4-methoxy-1-naphthol and hydrogen peroxide. Antigen-positive plaques were picked up and stored in 0.1 M NaCl–10 mM $MgSO_4$–50 mM Tris-HCl (pH 7.5)–0.01% gelatin containing a drop of chloroform. Liquid lysates of the recombinant phages, prepared as described previously (17), were used for immunoblot analysis.

SDS-PAGE and Western immunoblot analyses. The liquid lysates of the recombinant phages and *E. coli* containing recombinant plasmids were denatured by heating at 100°C for 5 min in a sample buffer containing 1% 2-mercaptoethanol and 2% SDS. The samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) as described by Laemmli (14). Western blot analysis was performed as previously described (13). The blocking and subsequent incubations were conducted in the same manner as described for plaque screening, except that incubations with first antibodies were carried out overnight. Human sera, used as antibodies for immunoblotting analysis, were diluted 1:100.

Isolation of recombinant DNA and subcloning. DNAs of the recombinant phages and colonies were isolated by a liquid lysate method and an alkaline lysis method, respectively (17). DNA fragments of the recombinant phage were subcloned in plasmid vector pUC18 with *E. coli* XL1-Blue.

DNA hybridization. For dot-blot analyses, genomic DNAs (500 ng) were applied to nylon membranes (Hybond-N+; Amersham) by using a Bio-Dot blotting apparatus (Bio-Rad Laboratories, Richmond, Calif.). For Southern blot analysis, genomic DNAs were digested with three restriction endonucleases (*Hin*dIII, *Pst*I, and *Eco*RV), electrophoresed on an agarose gel, and blotted onto nylon membranes by using a VacuGene blotting system (Pharmacia LKB Biotechnology, Uppsala, Sweden). DNA probes for the hybridization were prepared from a restriction fragment of the recombinant plasmid labeled with [α-^{32}P]dCTP by the random primer DNA labeling method (6). Hybridization with the DNA probe was carried out in a hybridization buffer as described by Church and Gilbert (4). After hybridization for 16 h at 65°C, the membranes were washed twice at 55°C with a solution containing 30 mM NaCl–3 mM sodium citrate (pH 7.0)–0.1% SDS. The membranes were dried and autoradiographed.

Nucleotide sequencing. For nucleotide sequence determination, 15 restriction fragments (9 for the sense strands and 6 for the antisense strands) that cover the entire cloned region were subcloned into M13mp18, M13mp19, or Phagescript SK (Stratagene, San Diego, Calif.) with *E. coli* XL1-Blue. The single-stranded template DNAs were isolated by the procedure of Messing (19). Nucleotide sequences were determined by the dideoxy chain termination method of Sanger et al. (22) with the Sequenase kit (U.S. Biochemical Corp., Cleveland, Ohio) with the standard M13 primer and synthetic oligonucleotides. Both DNA and deduced amino acid sequences were analyzed by using computer programs (DNASIS; Hitachi Software Engineering Corp., Tokyo, Japan).

Nucleotide sequence accession number. The sequence data in Fig. 3 will appear in DDBJ, EMBL, and GenBank nucleotide sequence data bases under accession number D00598.

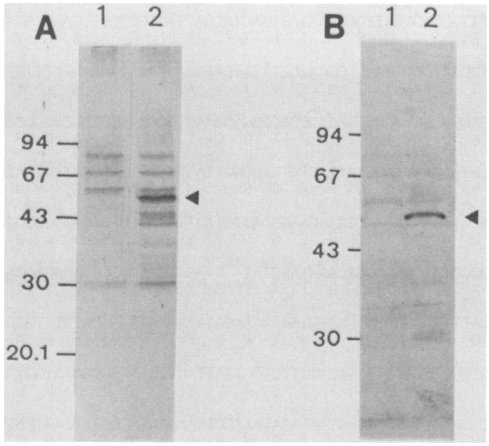

FIG. 1. Western blot analyses of the recombinant proteins. (A) Lanes: 1, EMBL3 lysate as a control; 2, lysate of the recombinant phage clone λTD1. (B) Lanes: 1, *E. coli* with pUC18 as a control; 2, *E. coli* with subclone pTD103. Numbers on the left indicate molecular mass in kilodaltons. The positions of the 53-kDa protein (TdpA) are indicated by the arrowheads.

RESULTS

Isolation of a clone encoding treponemal antigen. A genomic library of *T. denticola* was constructed with a bacteriophage replacement vector, EMBL3. The packaging efficiency with *E. coli* LE392 was approximately 1.0×10^4 PFU/μg of DNA, similar to that with the *E. coli* P2-lysogenic strain Q359, indicating that almost all plaques consisted of recombinant DNA phages (7). Among 1.0×10^5 plaques we isolated 2, designated λTD1 and λTD2, that exhibited the most intensive signal when the library was screened with the rabbit antiserum against whole cells of *T. denticola*. No significant differences were observed in the SDS-PAGE profiles between these recombinant phage lysates and an EMBL3 control lysate (data not shown), as detected by protein staining with Coomassie brilliant blue. However, among the Western blots of proteins, several protein bands immunologically stained were determined to be specific for the recombinant phage lysate. One major band was seen at 53 kDa in the λTD1 lysate (Fig. 1A, lane 2). The Western blot profile of the λTD2 lysate was essentially the same as that of the λTD1 lysate; therefore, both clones are concluded to encode the 53-kDa antigen. We designated the recombinant protein as *T. denticola* protein A (TdpA), and we designated the gene encoding the protein as *tdpA*.

Subcloning of *tdpA*. Figure 2A shows a restriction map of the recombinant λTD1 clone, obtained by partial restriction endonuclease analysis. The clone contains *T. denticola* DNA of 2.1 kb between the *Bam*HI sites of EMBL3. A 2.1-kb *Sal*I-*Eco*RI fragment containing *T. denticola* DNA was subcloned into plasmid vector pUC18 with *E. coli* XL1-Blue to create a recombinant plasmid, pTD103. The sequence analysis revealed no stop codon in the insert of pTD103, indicating the absence of the C-terminal region of *tdpA*. We obtained the remaining C-terminal region by screening an M13mp18 sublibrary containing the C-terminal region of *tdpA* in a 2.3-kb *Pst*I fragment (Fig. 2). Finally, we constructed pTD621, containing the entire coding region of *tdpA*, by joining the 5′ fragment to the 3′ fragment at the *Pst*I site in the coding region. Restriction maps of the subclones and the corresponding genomic DNA are shown in Fig. 2C. Western blot analysis showed that the *E. coli* clone carrying pTD621 produced the 53-kDa protein, TdpA (data not shown). No significant difference was observed between the Western blot profiles of clones containing pTD103 (Fig. 1B, lane 2) and pTD621.

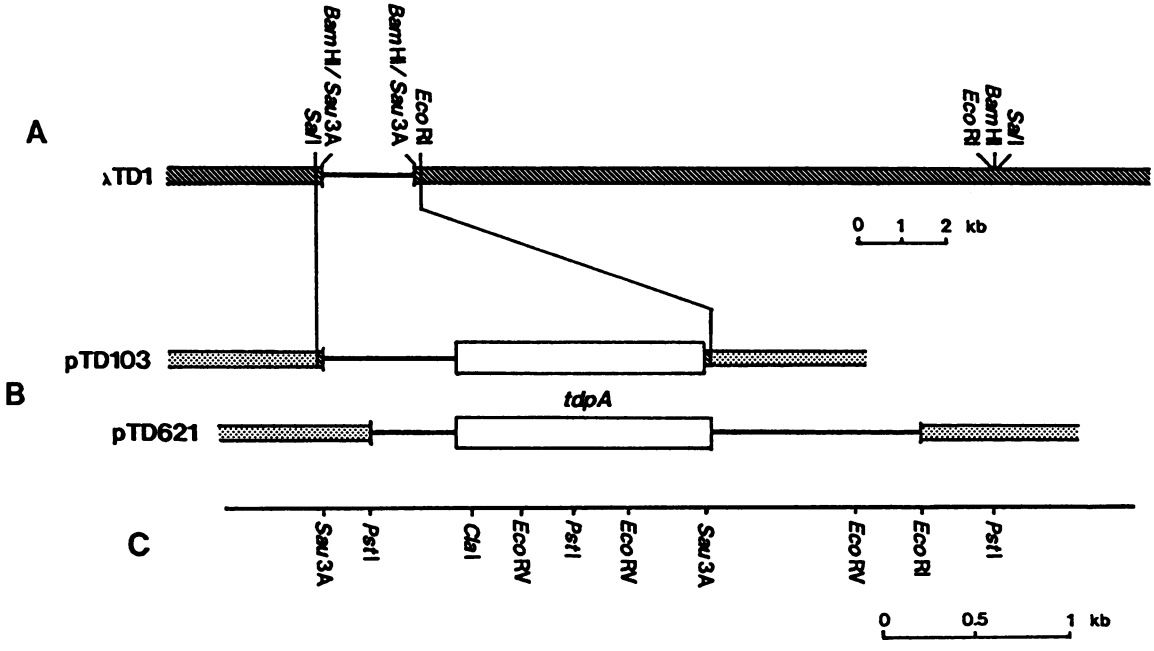

FIG. 2. Restriction maps of λTD1, pTD103, and pTD621 containing *tdpA*. (A) Recombinant phage λTD1; (B) recombinant plasmids pTD103 and pTD621; (C) restriction map of the corresponding genomic DNA fragment of *T. denticola* Johnson. Symbols: ▨, EMBL3 DNA; ▩, pUC18 DNA; ▭, region encoding *tdpA*.

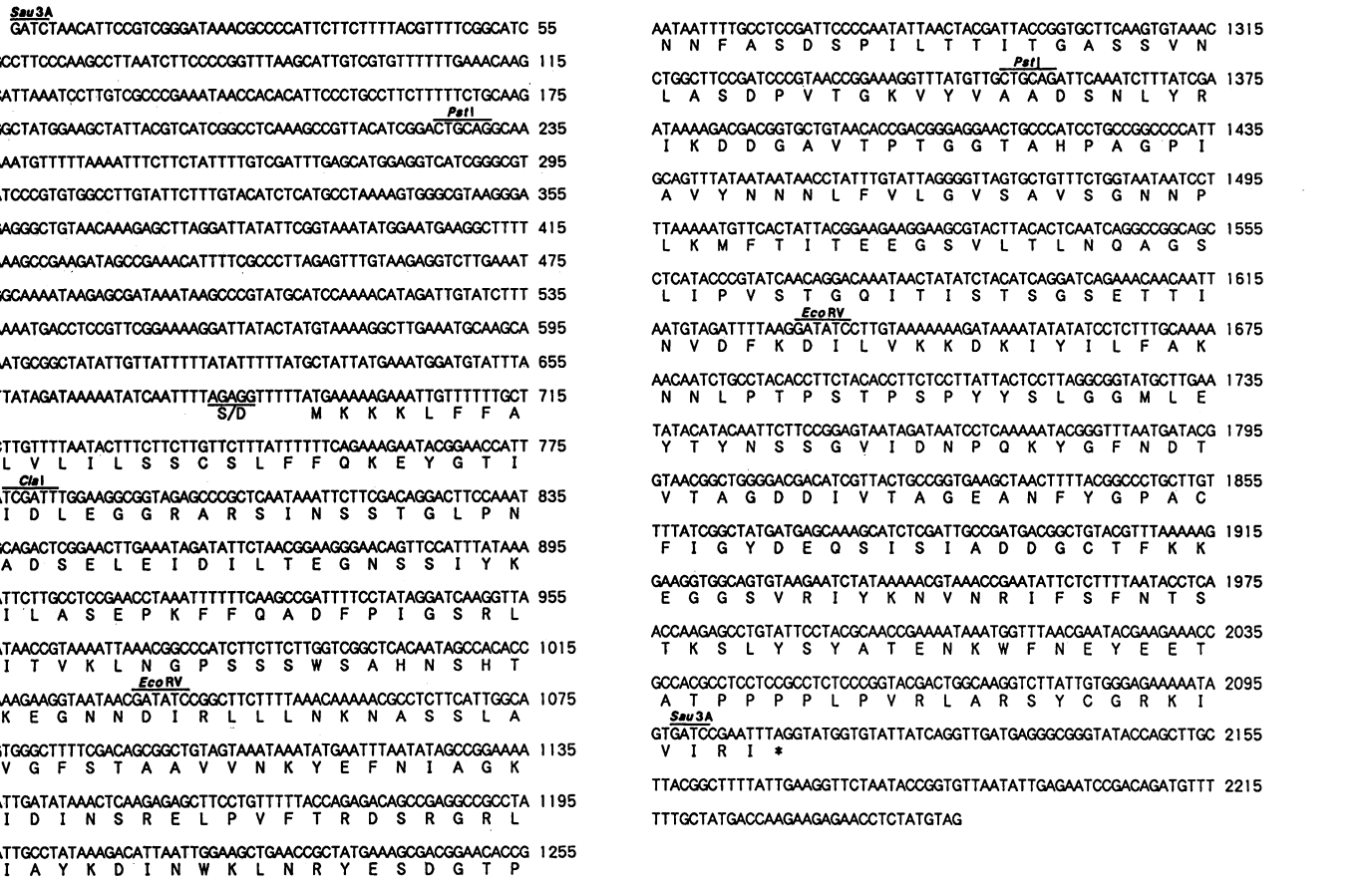

FIG. 3. Nucleotide sequence of *tdpA* and the deduced amino acid sequence of the TdpA protein. Numbers beside the sequences refer to nucleotide positions. A possible ribosome-binding site (S/D) is underlined. The locations of the endonuclease digestion sites are indicated along the sequence.

Nucleotide sequence of *tdpA*. The entire sequence of *tdpA* is shown in Fig. 3. The only possible open reading frame of *tdpA* was assigned from the ATG codon at position 692 to the stop codon at position 2110 (Fig. 3). The open reading frame consists of 1,419 bp, coding a putative protein with 472 amino acids (molecular weight, 51,427). The hydrophobicity plot of TdpA analyzed with the algorithm of Hopp and Woods (9) (Fig. 4) indicates a prominent hydrophobic domain at the N-terminal region. The N-terminal sequence was found to be homologous with a signal sequence which consists of three charged residues (Lys) followed by a hydrophobic core region. To predict the biological function of TdpA, the amino acid sequence of TdpA was subjected to a homology search with the NBRF protein sequence data base; no significant sequence homology was found.

In the 5′-noncoding region, a sequence, AGAGG, 5 bp

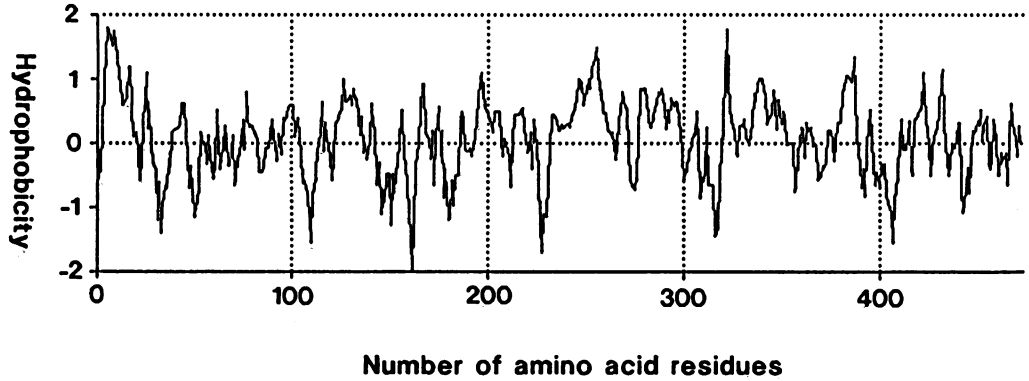

FIG. 4. Hydrophobicity of the TdpA antigen protein, analyzed by the method of Hopp and Woods (9). Values for hydrophobicity were calculated across six amino acid residues. Note the prominent hydrophobic domain at the N-terminal part of the molecule.

TABLE 2. Codon usage in the *tdpA* gene and *E. coli* genes

Amino acid	Codon	Codon frequency in[a]:	
		tdpA	*E. coli*[b]
Ala	GCU	23	20
	CCC	27	23
	GCA	15	21
	GCG	2	35
Arg	CGU	0	29
	CGC	6	22
	CGA	10	2
	CGG	2	3
	AGA	13	1
	AGG	4	1
Asn	AAU	47	13
	AAC	32	27
Asp	GAU	32	30
	GAC	19	25
Cys	UGU	8	4
	UGC	0	6
Gln	CAA	8	12
	CAG	4	32
Glu	GAA	42	47
	GAG	4	19
Gly	GGU	17	32
	GGC	23	31
	GGA	25	4
	GGG	11	8
His	CAU	2	9
	CAC	4	12
Ile	AAU	27	24
	AUC	19	32
	AUA	34	2
Leu	UUA	21	8
	UUG	6	10
	CUU	27	8
	CUC	8	9
	CUA	6	2
	CUG	11	59
Lys	AAA	49	39
	AAG	13	12
Met	AUG	6	27
Phe	UUU	42	16
	UUC	4	20
Pro	CCU	30	5
	CCC	6	3
	CCA	6	7
	CCG	8	26
Ser	UCU	32	12
	UCC	17	11
	UCA	21	5
	UCG	11	7
	AGU	8	6
	AGC	15	15
Thr	ACU	11	11
	ACC	21	25
	ACA	27	5
	ACG	17	11
Trp	UGG	6	11
Tyr	UAU	30	13
	UAC	17	15
Val	GUU	17	24
	GUC	0	13
	GUA	36	13
	GUG	4	24

[a] The codon frequency is given as the number of codons used per 1,000 codons.
[b] Data from reference 18.

upstream from the methionine initiation codon may be a ribosomal binding site (23). The consensus sequences for bacterial promoters cannot be assigned yet in the expectable regions; however, some A+T-rich regions in the noncoding sequence are presumed to function as a promoter for expression of *tdpA* in *E. coli*, because *tdpA* was actually expressed in an EMBL3 recombinant in which no specific bacterial promoter is designed. No explicit transcriptional termination signal was found in the sequence.

Table 2 summarizes our analysis of the codon usage in *tdpA*. For comparison, an average frequency of codons used in *E. coli* genes (18) is also included. The codon usage in *tdpA* differs significantly from that in *E. coli* genes. For example, the isoleucine codon AUA and the glycine codon GGA are used frequently in *tdpA*, but they are rarely used in *E. coli*. The G+C content was estimated to be 40% in the coding region of *tdpA*, in agreement with a reported G+C content of 37 to 38% for *T. denticola* (24).

Sequence homology of *tdpA* with other bacterial DNAs. A DNA hybridization analysis was performed to search for homology of *tdpA* with other bacterial chromosomal DNAs, including spirochete DNAs. Figure 5 shows results obtained by slot blot analysis with the 1.4-kb *Cla*I-*Eco*RI fragment as a probe. The probe hybridized intensively with DNA from each strain of *T. denticola* species but rather moderately with DNAs from three strains of other oral *Treponema* species. No hybridization was observed for the DNAs from other spirochetes, *E. coli*, or oral gram-negative rods.

We performed Southern blot hybridization analysis on restriction fragments of genomic DNAs from four spirochetes (Fig. 6). We observed 1.1- and 2.3-kb hybridizing bands for *T. denticola* Johnson for *Pst*I digests (Fig. 6, lane A) and a 6.5-kb band for *T. denticola* ATCC 35405 (lane B). Although the *Pst*I digestion profile of *T. denticola* Johnson is the same as that of *T. denticola* ATCC 33520, the digestion patterns with *Eco*RV differ. These results indicated that the *tdpA* gene exhibits remarkable polymorphism, at least in the four *Treponema* strains.

Serological responses to TdpA in periodontitis patients. To determine whether human serum contains antibodies against TdpA, the *E. coli* extract containing the recombinant TdpA was examined in an immunoblot with the human sera from normal subjects and periodontitis patients. Although several

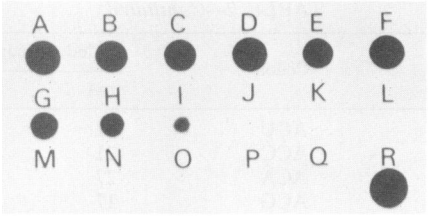

FIG. 5. Slot blot hybridization analysis of genomic DNAs of various bacteria with a probe for *tdpA*. *T. denticola* strains: A, Johnson; B, ATCC 35405; C, ATCC 35404; D, ATCC 33520; E, S-2; F, OKA-3. *Treponema* species strains: G, E-21; H, E-30; I, Y-181; J, G7201. *T. socranskii* strains: K, ATCC 35534; L, ATCC 35536. Other species: M, *T. pallidum* ATCC 27087; N, *P. gingivalis* 381; O, *A. actinomycetemcomitans* ATCC 29523; P, *W. recta* ATCC 33238; Q, *E. coli* LE392. R, recombinant plasmid (10 pg) as a positive control.

bands for *E. coli* proteins were observed, the bands for TdpA were intensively detected in Fig. 7, lane 7, and weakly in other lanes. This result indicates that the sera from some periodontitis patients contain antibodies against TdpA. However, Western blot profiles with the sera from periodontitis patients to *T. denticola* lysate showed that the 53-kDa protein is not a major protein (data not shown). Further investigation is necessary to elucidate correlations between the presence of the antibody against the antigen and the state of periodontal disease.

DISCUSSION

We report here the successful isolation of the gene that encodes an immunogenic protein of *T. denticola*, and we demonstrate that the cloned antigen gene is common in *T. denticola* isolates, despite showing considerable polymorphism. Furthermore, three strains of other oral *Treponema* species, E-21, E-30, and Y-181, were demonstrated to possess a gene(s) homologous to *tdpA*. Of particular interest is

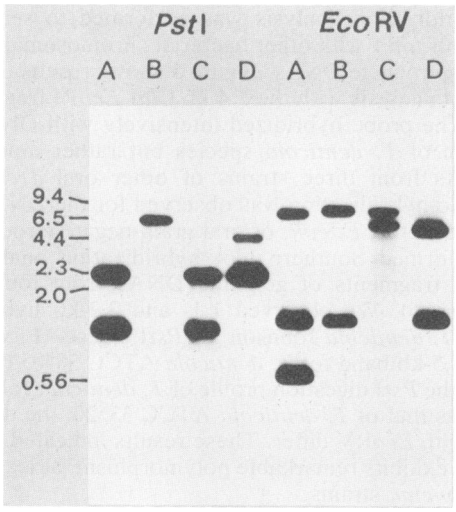

FIG. 6. Southern blot hybridization analysis of genomic DNAs of oral spirochetes with a probe for *tdpA*. Lanes: A, *T. denticola* Johnson; B, *T. denticola* ATCC 35405; C, *T. denticola* ATCC 33520; D, *Treponema* species strain E-21. Numbers on the left indicate molecular sizes in kilobase pairs.

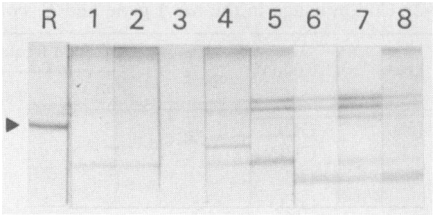

FIG. 7. Western blot analysis of the TdpA antigen with human sera. Lanes: R, rabbit antiserum; 1 and 2, sera from healthy donors; 3 through 8, sera from periodontitis patients. The arrowhead indicates the TdpA antigen.

that their morphological and biochemical features resembled those of *T. denticola* (1, 12, 27). In fact, a 2-4-2 pattern in the arrangement of their periplasmic flagella was revealed by the electron microscopic studies, and their production of indole, hydrogen sulfide, and liquefied gelatin was shown by biochemical analysis. In contrast, *Treponema* sp. strain G7201, whose chromosomal DNA did not hybridize with the probe for *tdpA*, is closely similar to *Treponema macrodontium*, rather than to *T. denticola*, in terms of fermentation patterns and morphological features (28). Thus, the *tdpA* gene should be a useful probe for in situ or in vitro identification of *T. denticola* in clinical samples from subgingival plaques.

Although the function of the TdpA antigen has not yet been elucidated, the presence of a signal peptide sequence indicates that it is a membrane-bound or periplasmic protein. The sequence Leu-Ser-Ser-Cys, amino acid residues 14 through 17, is one of the consensus sequences, Leu-X-Y-Cys, assigned as recognition sites of signal peptidase II (32). Since signal peptidase II cleaves the signal sequence before the cysteine residue, the putative signal peptide from methionine to the Ser-16 of the antigen may be removed to form the mature antigen composed of 456 amino acids with a molecular weight of 49,594. A recognition site for signal peptidase II has been reported to be characteristic, in general, of a bacterial lipoprotein (32), suggesting that the TdpA antigen is a lipoprotein. Swancutt et al. (25, 26) revealed that the sequence of the 34-kDa lipoprotein of *T. pallidum* contains a consensus signal peptidase II recognition site. The published sequence of the other membrane protein of *T. pallidum*, TmpA (8), contains a similar sequence. It is interesting that the sequences of immunogenic membrane proteins of treponemes contain signal peptidase II recognition sites in their signal sequences.

Several antigens similar to the TdpA protein have been reported: Umemoto et al. (29) reported a major antigen of *T. denticola*, with a molecular weight of 53,000, localized on the surface of the outer envelope. They also reported that the "53-kDa antigen" reacted strongly with rabbit antiserum against *T. denticola* ATCC 33520 and also with sera from periodontitis patients (30). However, the TdpA antigen seems to be distinct from theirs because their immunoblot analysis showed that both polyclonal and monoclonal mouse antibodies against the "53-kDa antigen" of *T. denticola* ATCC 33520 did not react with the cell lysates from any other strains of *T. denticola* (29). Cockayne et al. (5) demonstrated that antiserum raised against whole cells of *T. denticola* ATCC 33520 recognized polypeptides of 72, 68, 54, and 52 kDa. Since their 54-kDa polypeptide appeared to be a breakdown product of a larger (5), heat-modifiable polypeptide, it appears to be different from TdpA. On the other hand, we frequently observed a 52-kDa band in Western blots of various *T. denticola* strains with the rabbit antiserum

used to detect the recombinant TdpA from *E. coli* (data not shown). Thus, the 52-kDa antigen may be a processed form of TdpA in *T. denticola*.

In conclusion, we isolated a gene encoding an antigen apparently specific for *T. denticola*. The coding region of the gene is proved to be useful as a DNA probe for identification of *T. denticola* and will be applicable for clinical examinations. The TdpA antigen may be a membrane-bound or periplasmic protein.

ACKNOWLEDGMENTS

We are grateful to T. Watanabe, T. Umemoto, and T. Eguchi for kindly providing bacterial strains. We also thank T. Nohno for introducing us to the DNA sequencing technique and M. Nakamura and T. Hatanaka for preparation of synthetic oligonucleotides.

This work was supported in part by grants 63771555 and 63480421, by Special Project Research (Okayama University, 1990) funds from the Ministry of Education, Science and Culture of Japan, and by the research fund from Yakult Co. Ltd.

REFERENCES

1. **Asai, A.** 1976. Studies on some morphological, biological and biochemical characteristics of the new cultivated oral spirochetes. Odontology **64**:232–252.
2. **Boehringer, H., N. S. Taichman, and B. J. Shenker.** 1984. Suppression of fibroblast proliferation by oral spirochetes. Infect. Immun. **45**:155–159.
3. **Bullock, W. O., J. M. Fernandez, and J. M. Short.** 1987. XL1-Blue: a high efficiency plasmid transforming recA *Escherichia coli* strain with beta-galactosidase selection. Biotechniques **5**:376–379.
4. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81**:1991–1995.
5. **Cockayne, A., R. Sanger, A. Ivic, R. A. Strugnell, J. H. Macdougall, R. R. B. Russell, and C. W. Penn.** 1989. Antigenic and structural analysis of *Treponema denticola*. J. Gen. Microbiol. **135**:3209–3218.
6. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
7. **Frischauf, A., H. Lehrach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170**:827–842.
8. **Hansen, E. B., P. E. Pedersen, L. M. Schouls, E. Severin, and J. D. A. Van Embden.** 1985. Genetic characterization and partial sequence determination of a *Treponema pallidum* operon expressing two immunogenic membrane proteins in *Escherichia coli*. J. Bacteriol. **162**:1227–1237.
9. **Hopp, T. P., and K. R. Woods.** 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA **78**:3824–3828.
10. **Jacob, E., and R. K. Nauman.** 1982. Common antigens of *Treponema denticola*: chemical, physical, and serological characterization. Infect. Immun. **37**:474–480.
11. **Karn, J., S. Brenner, L. Barnett, and G. Cesareni.** 1980. Novel bacteriophage λ cloning vector. Proc. Natl. Acad. Sci. USA **77**:5172–5176.
12. **Kato, M.** 1971. Some characters on 2 strains (E-30, E-31) of oral spirochetes. Odontology **59**:193–215.
13. **Kokeguchi, S., K. Kato, H. Kurihara, and Y. Murayama.** 1989. Cell surface protein antigen from *Wolinella recta* ATCC 33238^T. J. Clin. Microbiol. **27**:1210–1217.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature (London) **227**: 680–685.
15. **Leschine, S. B., and E. Canale-Parola.** 1980. Rifampin as a selective agent for isolation of oral spirochetes. J. Clin. Microbiol. **12**:792–795.
16. **Loesche, W. J., and B. E. Laughon.** 1982. Role of spirochetes in periodontal disease, p. 62–75. *In* R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Maruyama, T., T. Gojobori, S. Aota, and T. Ikemura.** 1986. Codon usage tabulated from the GenBank genetic sequence data. Nucleic Acids Res. **14**(Suppl.):r151–r197.
19. **Messing, J.** 1983. M13-dideoxy method. Methods Enzymol. **101**:20–78.
20. **Murayama, Y., A. Nagai, K. Okamura, H. Kurihara, Y. Nomura, S. Kokeguchi, and K. Kato.** 1988. Serum immunoglobin G antibody to periodontal bacteria. Adv. Dent. Res. **2**:339–345.
21. **Murray, N. E., W. J. Brammar, and K. Murray.** 1977. Lambdoid phages that simplify the recovery of *in vitro* recombinants. Mol. Gen. Genet. **150**:53–58.
22. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
23. **Shine, J., and L. Dalgarno.** 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71**:1342–1346.
24. **Smibert, R. M.** 1984. Order I. *Spirochaetales*, p. 49–57. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
25. **Swancutt, M. A., J. D. Radolf, and M. V. Norgard.** 1990. The 34-kilodalton membrane immunogen of *Treponema pallidum* is a lipoprotein. Infect. Immun. **58**:384–392.
26. **Swancutt, M. A., B. S. Riley, J. D. Radolf, and M. V. Norgard.** 1989. Molecular characterization of the pathogen-specific, 34-kilodalton membrane immunogen of *Treponema pallidum*. Infect. Immun. **57**:3314–3323.
27. **Takahashi, T.** 1968. Isolation and pure cultivation of oral spirochetes, and electron microscopic studies on the cell structure as well as the biological characters of pure cultivation strains. Odontology **56**:299–332.
28. **Umemoto, T., and I. Namikawa.** 1980. Electron microscopy of the spherical body of oral spirochetes *in vitro*. Further studies. Microbiol. Immunol. **24**:321–334.
29. **Umemoto, T., I. Namikawa, H. Suido, and S. Asai.** 1989. A major antigen on the outer envelope of a human oral spirochete, *Treponema denticola*. Infect. Immun. **57**:2470–2474.
30. **Umemoto, T., J. J. Zambon, R. J. Genco, and I. Namikawa.** 1988. Major antigens of human oral spirochetes associated with periodontal disease. Adv. Dent. Res. **2**:292–296.
31. **Van Embden, J. D., H. J. Van Der Donk, R. V. Van Eijk, H. G. Van Der Heide, J. A. De Jong, M. F. Van Olderen, A. D. Osterhaus, and L. M. Schouls.** 1983. Molecular cloning and expression of *Treponema pallidum* DNA in *Escherichia coli* K-12. Infect. Immun. **42**:187–196.
32. **Wu, H. C., and M. Tokunaga.** 1986. Biogenesis of lipoproteins in bacteria. Curr. Top. Microbiol. Immunol. **125**:127–157.