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# Common Antigens of *Treponema denticola*: Chemical, Physical, and Serological Characterization

ELLIOT JACOB AND ROBERT K. NAUMAN*

*Department of Microbiology, University of Maryland Dental School, Baltimore, Maryland 21201*

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A sodium deoxycholate-ethanol extractable antigen (DES-Ag) was obtained from four serotypes of *Treponema denticola* and characterized by chemical, physical, and serological procedures. The cross-reactivity of these antigens was demonstrated by indirect microhemagglutination, immunodiffusion, and immunoelectrophoresis with hyperimmune rabbit antiserum against each of the *T. denticola* serotypes. Antiserum against two nonoral treponemes, *T. phagedenis* biotype Reiter and *T. pallidum* Nichols strain, did not cross-react with the DES-Ag of *T. denticola*. Chemical analysis of the DES-Ag indicated that proteins (84%) and hexoses (12%) accounted for 96% of the total dry weight of the antigen. Trace amounts of *N*-acetylglucosamine (0.6%) were also detected. Fatty acids, including palmitic, oleic, and stearic, were identified by gas-liquid chromatography. Polyacrylamide gel electrophoresis of the DES-Ag of each serotype revealed the presence of two bands. The molecular weights of the bands were estimated to be 58,000 and 31,000 by comparing the electrophoretic mobility of the DES-Ag to that of five protein standards of known molecular weights. Although only a single precipitin line was observed by immunodiffusion when each antigen was reacted against its homologous antiserum, two precipitin lines were evident by immunoelectrophoresis. Antiserum against the DES-Ag of *T. denticola* was shown to agglutinate whole cells of the homologous serotype. Adsorption of this anti-DES-Ag serum with whole cells of *T. denticola* resulted in the elimination of precipitating antibodies to the DES-Ag by immunodiffusion. It is concluded that the DES-Ag is a component of the outer sheath of *T. denticola*.

The oral anaerobic spirochetes represent a diversified group of nutritionally fastidious microorganisms whose normal ecological niche is the subgingival dental plaque associated with the human gingival sulcus. The results of several previous investigations have indicated that there is a strong correlation between the percentage of spirochetes within a given plaque sample and clinical evidence of periodontal disease at the site from which the plaque specimen was obtained. Schultz-Haudt et al. (20) examined by light microscopy subgingival plaque taken from either healthy or periodontally involved patients and reported a 17% increase in the numbers of spirochetes in the plaque specimens of the patients with periodontal disease. Listgarten and Hellden (12) determined the relative distribution of bacteria at clinically healthy and periodontally diseased sites within the same individual and noted that whereas the spirochetes accounted for less than 2% of the total microscopic flora at the healthy sites, their proportions increased from 25 to 58% within the diseased sites. Slots et al. (21) examined the pretreatment plaque of six patients with periodontitis and reported that the spirochetes comprised 30% of the total microscopic flora. After a therapeutic regimen of scaling and root planing, the spirochetes were reduced to nondetectable levels and had not returned to base-line levels in 9 of 18 periodontal pockets even at 6 months posttherapy. A crucial finding of this study was that a correlation could be made between the appearance of soft tissue inflammation and an increase in the proportion of spirochetes within the subgingival plaque. Although these studies do not provide direct evidence implicating spirochetes as etiological agents of periodontal disease, they do demonstrate the fact that the periodontal pocket provides a suitable ecological niche for spirochetal proliferation.

Our laboratory has isolated several strains of *Treponema denticola* from the subgingival plaque of clinic patients with advanced periodontitis. In a previous study, we have shown that these isolates differ with respect to surface antigenic determinants and may be considered as representing individual serotypes (11). We have also reported on the isolation of a sodium deoxycholate-ethanol extractable antigen (DES-

Ag) from *T. denticola* and its serological reactivity with the sera of patients with advanced periodontitis (11a). The purpose of the present investigation was to characterize these antigens of *T. denticola* by using chemical, physical, and serological techniques.

## MATERIALS AND METHODS

**Bacterial strains.** The four serotypes of *T. denticola* used in this investigation were W (now ATCC 33520), 11 (now ATCC 33521), 14, and TT. They were originally isolated from the subgingival plaque of patients with advanced periodontal disease by the membrane filter technique of Loesche and Socransky (13). Stock cultures of each isolate were maintained on semisolid medium consisting of PPLO broth (BBL Microbiology Systems, Cockeysville, Md.) plus 5 μg of cocarboxylase per ml, 10% sterile rabbit serum, and 0.2% ion agar. The same medium lacking ion agar was employed for liquid cultivation. Electron microscopic studies of negatively stained specimens indicated that all of the isolates were identical morphologically and possessed the "2-4-2" axial filament arrangement. Gas-liquid chromatographic analysis of culture supernatant fluids indicated metabolic fatty acid end products, including acetic, propionic, and succinic acids, which are characteristic for *T. denticola*.

**Antiserum preparation.** Hyperimmune rabbit antiserum was prepared against each serotype of *T. denticola* in New Zealand white rabbits as described previously (10). Rabbit antisera to *T. phagedenis* biotype Reiter and *T. pallidum* Nichols strain were obtained from E. F. Hunter, Centers for Disease Control, Atlanta, Ga., and J. B. Baseman, University of Texas at San Antonio, respectively.

**Antigen extraction.** A sodium deoxycholate-ethanol extraction procedure, similar to that described by Chang and McComb (5), was used to obtain antigens from each isolate of *T. denticola*. Organisms from 20 ml of an actively growing broth culture were harvested by centrifugation at 10,000 × *g* for 30 min. The sedimented cells were suspended in 0.85% NaCl to an optical density of 0.25 at a wavelength of 650 nm in a Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). A 0.5-g amount of sodium deoxycholate was added per 10 ml of cell suspension, and the mixture was incubated at 37°C for 2 h. Cold absolute ethanol was then added to a final concentration of 90%, and the mixture was kept at 4°C overnight. The resulting precipitate was collected by centrifugation at 12,000 × *g* for 30 min. The precipitate was suspended in 10 ml of 0.15 M phosphate-buffered saline (PBS) (pH 7.2) containing 0.02% sodium azide and was designated as the deoxycholate-ethanol spirochetal antigen (DES-Ag) preparation.

**Indirect hemagglutination assay.** The indirect hemagglutination assay procedure of Boyden (3) was used. The optimal dilution of DES-Ag needed to sensitize tannic acid-treated sheep erythrocytes was 16 hemagglutination units, as determined previously by a dual titration of each antigen against its homologous rabbit antiserum (11). To determine whether the DES-Ag was shared by the various serotypes of *T. denticola*, tannic acid-treated sheep erythrocytes were each sensitized with 16 hemagglutination units of DES-Ag extracted from serotypes W, 14, 11, and TT and reacted against serial twofold dilutions of the homologous and heterologous rabbit antisera in dilutions ranging from 1:10 to 1:2,560. Studies were also performed to determine whether these antigens were shared with nonoral treponemes. The sodium deoxycholate-ethanol extraction procedure was used to obtain antigens from *T. phagedenis* biotype Reiter. Tanned erythrocytes were sensitized with 16 hemagglutination units of antigen extracted from *T. phagedenis* biotype Reiter and reacted with serial twofold dilutions, ranging from 1:10 to 1:2,560, of the following rabbit antisera: anti-*T. phagedenis* biotype Reiter; anti-*T. denticola* W, 11, 14, and TT; and anti-*T. pallidum* Nichols. All of the antisera were previously heat inactivated at 56°C for 30 min and adsorbed with a 10% suspension of sheep erythrocytes. Controls included a 1:10 dilution of each antiserum reacted with tanned nonsensitized erythrocytes as well as sensitized erythrocytes reacted with the diluent (0.3% gelatin in PBS [pH 7.2]) instead of antiserum. Agglutination patterns were recorded after a 2-h incubation period at 25°C on a 1 to 4+ basis, with 4+ indicating a mat or carpet of cells displaying the highest degree of agglutination. The highest dilution of each antiserum displaying at least a 2+ agglutination pattern was recorded as the endpoint.

**ID and IE.** Immunodiffusion (ID) tests were performed on microscope slides with 0.85% agarose in PBS (pH 7.2) as the diffusion medium by the method of Ouchterlony (18). The optimal conditions for immune precipitation were assessed by charging the peripheral wells with serial twofold dilutions of DES-Ag containing protein concentrations ranging from 61.0 to 3.8 μg and adding the undiluted homologous rabbit antiserum to the center well. The slides were placed in a moist chamber and kept at 25°C until precipitin lines became evident (usually 24 to 48 h). Once the optimal concentration of antigen had been determined, the ID test was repeated to determine whether rabbit antiserum prepared against one serotype of *T. denticola* would cross-react with the DES-Ag of the heterologous serotypes.

Immunoelectrophoresis (IE) was performed by the method of Crowle (6) on microscope slides with 0.85% agarose in Veronal buffer (pH 8.6) as the electrophoretic medium. IE was carried out at 4°C in a Gelman IE chamber (Gelman Instrument Co., Ann Arbor, Mich.) with a Thomas model 21 power supply (A. H. Thomas Inc., Philadelphia, Pa.). DES-Ag from serotype W, 11, or TT was applied to separate slides (10 μl containing 61 μg of protein) and electrophoresed at 13 V/cm for 3.5 h. After electrophoresis, the homologous or the heterologous hyperimmune rabbit antiserum was applied to the troughs. The slides were kept in a moist chamber at 25°C until precipitin lines were visible (usually 24 h).

**Chemical analysis.** To determine the chemical composition of the DES-Ag of *T. denticola*, antigen was extracted from a 100-ml broth culture of serotype TT as described above. After lyophilization, the total dry weight was determined, and the antigen was suspended in 3.0 ml of PBS (pH 7.2). Portions of this preparation were used for the chemical analysis studies. Protein was determined by the method of Lowry et al. (14) with bovine serum albumin as the standard. Total hexose content was analyzed by the method of Dische et al. (8) with glucose as the standard. *N*-Acetylgluco-

TABLE 1. Antibody titers of homologous and heterologous rabbit antisera to the DES-Ag extracted from four serotypes of *T. denticola* as determined by indirect microhemagglutination

| Antigen | Rabbit antiserum titer[a] | | | |
|---|---|---|---|---|
| | Anti-W | Anti-14 | Anti-11 | Anti-TT |
| W | 1,280 | 320 | 80 | 160 |
| 14 | 320 | 1,280 | 160 | ND[b] |
| 11 | 80 | 160 | 1,280 | 80 |
| TT | 160 | 160 | 80 | 1,280 |

[a] Reciprocal of highest serum dilution resulting in at least a 2+ agglutination pattern.
[b] Not determined.

samine was determined by the method of Aminoff et al. (1) with *N*-acetyl-D-glucosamine as the standard. Pentose was determined by the method of Brown (4) with ribose as the standard. Methylpentose content was determined by the method of Dische and Shettles (7) with rhamnose as the standard. Fatty acid content was analyzed by gas-liquid chromatography by the procedure of Moss et al. (16) with a Hewlett-Packard model 5710 A gas chromatograph.

**PAGE.** To determine whether the DES-Ag of *T. denticola* is composed of single or multiple components, polyacrylamide gel electrophoresis (PAGE) was performed by the method of Spear and Roizman (22). Seven percent polyacrylamide gels were prepared in 100 ml of 0.375 M Tris-hydrochloride buffer (pH 8.8) by using the following formula: acrylamide, 7 g; *N,N*-methylenebisacrylamide, 0.19 g; *N,N,N,N*-tetramethylethylenediamine, 30 μl (vol/vol); and ammonium persulfate, 0.1 g.

Approximately 2.0 ml of the gel solution was added to electrophoresis glass tubes (length, 75 mm; inner diameter, 5 mm), and photopolymerization of the gels was accomplished with a fluorescent light assembly containing two 15-W lamps. After polymerization, the DES-Ag, containing 50 μg of protein, of serotype W, 11, or TT was applied to the tops of separate gels, and electrophoresis was carried out in a Buchler Polyanalyst electrophoresis chamber (Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N.J.) and a Thomas model 21 power supply (A. H. Thomas Inc.). The electrode buffer was 0.025 M Tris-hydrochloride–glycine containing 0.015% bromophenol blue as a tracking dye. Electrophoresis was performed at a constant current of 2 mA per gel until the tracking dye had migrated approximately 70 mm from the origin of the gels. The gels were fixed overnight in a solution of 40% methanol and 7% glacial acetic acid. The gels were then stained overnight in a solution of 10% glacial acetic acid and 25% isopropanol containing 0.04% Coomassie brilliant blue R. Destaining was accomplished with several changes of 10% glacial acetic acid over a period of 2 to 3 days.

The molecular weight of the DES-Ag of *T. denticola* was estimated with PAGE by comparing the electrophoretic mobility of the DES-Ag with that of the following protein standards of known molecular weights: bovine albumin, 66,000; egg albumin, 45,000; pepsin, 34,700; trypsinogen, 24,000; and beta-lactoglobulin, 18,400. The molecular weight standards were purchased from Sigma Chemical Co., St. Louis, Mo.

## RESULTS

**Indirect microhemagglutination.** The indirect hemagglutination assay antibody titers of both the homologous and heterologous rabbit antisera when reacted with the DES-Ag of each *T. denticola* serotype are presented in Table 1. With the homologous reactions, the titers were 1:1,280 in all cases; with the heterologous reactions, the titers ranged from 1:80 to 1:320. The degree of cross-reactivity observed with the heterologous antisera indicated that the DES-Ag was shared by these four serotypes. The results presented in Table 2 show that these antigens are specific for the oral isolates of *T. denticola* and are not shared with either *T. phagedenis* biotype Reiter or *T. pallidum* Nichols. As can be seen from the data, rabbit anti-*T. phagedenis* biotype Reiter or anti-*T. pallidum* serum failed to agglutinate tanned erythrocytes sensitized with the DES-Ag of *T. denticola* serotype W, 11, or 14. Conversely, rabbit antiserum to each serotype of *T. denticola* or *T. pallidum* did not agglutinate tanned erythrocytes sensitized with a sodium deoxycholate-ethanol extract of *T. phagedenis* biotype Reiter.

**Cross-reactivity of DES-Ag by ID and IE.** The optimal conditions for ID were assessed by charging the peripheral wells of 0.85% agarose slides with serial twofold dilutions of DES-Ag with protein concentrations ranging from 61 to 3.8 μg and adding the homologous rabbit antiserum to the center well. With each of the homologous reactions, a single precipitin line was observed at DES-Ag protein concentrations of 61, 30.5, and 15.2 μg. No precipitation was observed at any of the other protein concentrations. All subsequent ID tests were performed with the DES-Ag at a protein concentration of 61 μg. The ID test was then repeated to determine whether rabbit antiserum prepared to one serotype would cross-react with the DES-Ag of the heterologous serotypes. DES-Ag extracted from serotypes W, 11, and TT was applied in duplicate to the peripheral wells, and rabbit antiserum against serotype W, 11, or TT was added to the central well. Figure 1 shows the results of

TABLE 2. Specificity of the DES-Ag for oral isolates of *T. denticola* as demonstrated by indirect microhemagglutination

| Antigen | Rabbit antiserum titer[a] | | |
|---|---|---|---|
| | *T. denticola*[b] | *T. phagedenis* | *T. pallidum* |
| *T. denticola*[c] | 1,280 | 0 | 0 |
| *T. phagedenis* | 0 | 640 | 0 |

[a] Reciprocal of highest serum dilution resulting in at least a 2+ agglutination pattern.
[b] Antiserum to serotype W, 11, or 14.
[c] DES-Ag extracted from serotype W, 11, or 14.

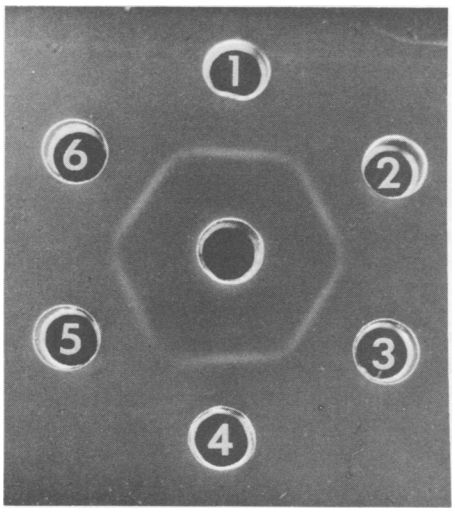


FIG. 1. Cross-reactivity of the DES-Ag from *T. denticola* as demonstrated by ID. The peripheral wells were charged with DES-Ag from serotype W (1 and 4), serotype 11 (2 and 5), and serotype TT (3 and 6). The central well was charged with rabbit antiserum against serotype W.

the ID test when antiserum to serotype W was reacted with the DES-Ag of three other serotypes. A single precipitin line was obtained with each of the antigens. The fact that these precipitin lines formed lines of identity indicated that the DES-Ag was shared by the various serotypes.

To investigate the possibility that the *T. denticola* DES-Ag may contain additional antigenic determinants that could not be detected by ID, IE was performed. Antigens extracted from serotype W, 11, or TT, containing 61 μg of protein, were subjected to electrophoresis for 3.5 h. After electrophoresis, the undiluted homologous rabbit antiserum was applied to the peripheral troughs. It can be seen in Fig. 2 that two separate precipitin lines were observed with the homologous antigen-antibody systems by IE. Identical results were obtained when each DES-Ag preparation was reacted against the heterologous rabbit antiserum by IE.

**Chemical analysis of the DES-Ag of *T. denticola*.** The results of the chemical analysis performed on the DES-Ag extracted from serotype TT are presented in Table 3. Proteins (84%) and carbohydrates in the form of hexoses (12%) accounted for approximately 96% of the total dry weight of the antigen. Trace amounts of *N*-acetylglucosamine (0.6%) were also present. Other carbohydrates, including pentose and methylpentose, were not detected. Gas-liquid chromatographic analysis of the antigen revealed the presence of fatty acids, including palmitic, stearic, and oleic acids.

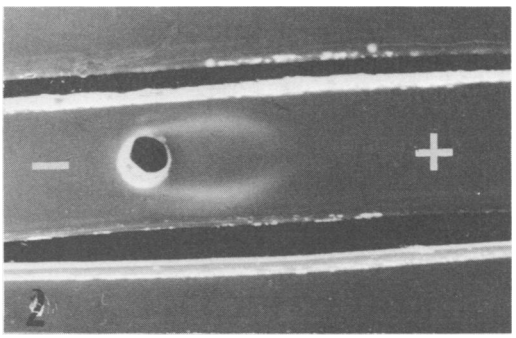


FIG. 2. Immunoelectrophoretic analysis of the DES-Ag from *T. denticola* serotype TT showing the presence of two separate precipitin lines. The DES-Ag was electrophoresed at 13 V/cm for 3.5 h, followed by the addition of the homologous rabbit antiserum to the troughs.

**PAGE.** To determine whether the DES-Ag was composed of single or multiple components, electrophoresis was carried out in 7% polyacrylamide gels. Figure 3 shows the results of PAGE with DES-Ag extracted from serotypes TT and 11. Typically, two major bands with absolute mobilities of 25 and 50 mm, respectively, were detected. Identical results were obtained with DES-Ag extracted from serotype W. The approximate molecular weight of each of these bands was estimated by the method of Zwann (25) by comparing the electrophoretic mobility of each band with that of five protein standards of known molecular weights. The molecular weight of the band at 25 mm was estimated to be 58,000, and that of the band at 50 mm was estimated to be 31,000.

**Antigen localization.** To determine whether the DES-Ag was a surface or internal component of the treponemal cell, hyperimmune rabbit antiserum was prepared against the DES-Ag of *T. denticola* serotype TT and reacted against whole cells of the homologous serotype by microscopic agglutination (MA). Serial twofold dilutions of this antiserum, ranging from 1:5 to 1:5,120, were prepared with 0.85% NaCl as the

TABLE 3. Chemical composition of DES-Ag extracted from *T. denticola* serotype TT

| Component | Content (μg/mg of dry wt in DES-Ag (%) |
|---|---|
| Protein | 838 (83.8) |
| Hexose | 120 (12.0) |
| *N*-Acetylglucosamine | 6 (0.60) |
| Pentose | 0 (0) |
| Methylpentose | 0 (0) |
| Fatty acids[a] | |

[a] Palmitic, oleic, and stearic acids were identified by gas-liquid chromatography.

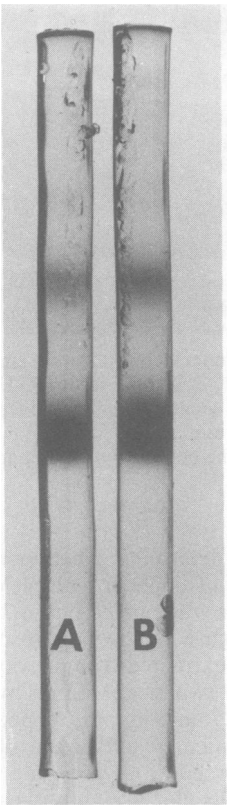


FIG. 3. PAGE of the DES-Ag from *T. denticola* serotype TT (A) and serotype 11 (B) showing two identical bands in each DES-Ag preparation.

diluent. An equal volume of an actively growing broth culture of serotype TT was added to 0.5 ml of each serum dilution and kept at 25°C for 2 h. Controls included the use of hyperimmune rabbit antiserum prepared against whole cells of serotype TT as well as nonimmune rabbit serum reacted against serotype TT. Whole cells were also reacted with saline instead of antiserum to evaluate the possibility of nonspecific agglutination. Agglutination patterns were recorded by using the 10× objective of a Leitz Dialux microscope equipped with a low-power dark-field condenser. The reciprocal of the highest serum dilution resulting in agglutination of equal to or greater than 50% of the spirochetal cells was designated as the antiserum titer. Antiserum prepared against whole cells of serotype TT had an MA titer of 1:1,280 against the homologous serotype; antiserum to the DES-Ag of serotype TT had an MA titer of 1:640 against whole cells of the same serotype. Nonspecific agglutination was not detected with any of the control reactions. The fact that antiserum prepared specifically against DES-Ag had the ability to agglutinate whole cells of *T. denticola* is a strong indication that this antigen may be a surface component of the cell.

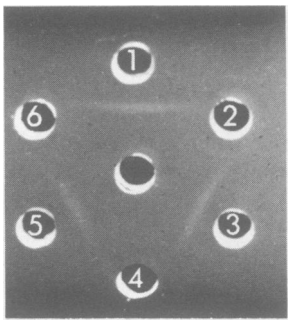


FIG. 4. Results of ID after adsorption of rabbit anti-DES-Ag serum with whole cells of *T. denticola* serotype TT. The peripheral wells were charged with either the unadsorbed (1, 3, and 5) or adsorbed (2, 4, and 6) antisera; the center well was charged with the DES-Ag from serotype TT.

Further verification that DES-Ag is located on the treponemal cell surface was obtained by two types of adsorption studies. In the first study, antiserum to the DES-Ag of serotype TT was heat inactivated at 56°C for 30 min and adsorbed with an equal volume of a broth culture of serotype TT. After a 1-h incubation period at 37°C, the cells were removed by centrifugation at 10,000 × *g* for 30 min, and the adsorbed antiserum was reacted against the DES-Ag of serotype TT by ID. The peripheral wells were charged in alternating sequence with either the adsorbed or unadsorbed antiserum; the central well was charged with the DES-Ag of serotype TT. Figure 4 shows that a single precipitin line was obtained with the unadsorbed serum; however, no precipitation was evident after the antiserum had been adsorbed with whole cells of serotype TT.

The second adsorption experiment was designed to investigate the possibility that during the process of adsorption, nonspecific lysis of the treponemal cells may have resulted in the liberation of internal DES-Ag that could have adsorbed the specific antibodies. To prevent lysis of the cells during the adsorption procedure, whole cells of serotype TT were prefixed in 1% glutaraldehyde at 4°C for 1 h. The cells were washed four times with PBS (pH 7.2) and were then used to adsorb the antiserum to the DES-Ag of serotype TT. Both the adsorbed and unadsorbed antisera were then reacted by ID against the DES-Ag of serotype TT. Whereas a single precipitin line was observed with the unadsorbed antiserum, no precipitation occurred after adsorption of the antiserum with the glutaraldehyde-fixed cells.

## DISCUSSION

Immunological studies of the oral spirochetes have been limited in scope primarily due to the difficulties encountered in the isolation and cultivation of these nutritionally fastidious, obligately anaerobic microorganisms. The few existing studies that deal with the antigenic composition of the oral spirochetes were originally undertaken to ascertain the relationship, if any, between the oral spirochetes and other members of the genus *Treponema*. In 1946, Robinson and Wichelhausen (19) described a method by which specific extracts may be prepared from spirochetes by dissolving the organisms in hot formamide followed by acid-alcohol precipitation. Various strains of oral spirochetes and nonpathogenic treponemes were differentiated with these extracts in a precipitation test with spirochetal immune sera. Nineteen oral spirochetes were classified into six serological groups on the basis of antigenic cross-reactivity of these extracts. A few years later, Eagle and Germuth (9) performed studies on the antigenic relationship between five nonoral treponemes (Noguchi, Kroo, Reiter, Nichols, and Kazan) and two oral treponemes. A common antigen was detected by both agglutination and complement fixation tests between the Reiter and Kazan treponemes and the two oral spirochetes. In 1966, Meyer and Hunter (15) examined the antigenic relationship among three strains of oral treponemes (MRB, FM, and N-39) by direct immunofluorescence. On the basis of reciprocal absorption studies, they reported that strains MRB and FM represent the same serotype, and strain N-39 represents a different serotype. In the same year, Nell and Hardy (17) reported on the extraction of a polysaccharide antigen from the Reiter treponeme which was shown to be shared by two oral treponemes (FM and PKOT), the N-9 strain of *Borrelia vincentii*, and three Kazan treponeme strains.

The present study describes the antigenic relationship of four strains of *T. denticola* originally isolated from the subgingival plaque of patients with advanced periodontitis. Although these isolates have previously been shown to represent individual serotypes (11), a sodium deoxycholate-ethanol extractable antigen (DES-Ag) is shared by these various strains, as demonstrated by indirect hemagglutination assay, ID, and IE. Moreover, these antigens appear to be specific for *T. denticola* and are not shared with either the Reiter treponeme or the Nichols strain of *T. pallidum*. Chemical analysis of the DES-Ag of *T. denticola* indicated that proteins (84%) and hexoses (12%) are the major chemical constituents present and account for approximately 96% of the total dry weight of the antigen. Trace amounts of *N*-acetylglucosamine (0.6%), a component of the peptidoglycan of bacterial cell walls, were also present. Fatty acids, including palmitic, oleic, and stearic acids, were identified by gas-liquid chromatography and presumably account for about 3% of the total dry weight. Electrophoretic analysis of the DES-Ag of each serotype in 7% polyacrylamide gels revealed the presence of two major bands. The molecular weight of the individual bands was estimated to be 58,000 and 31,000, respectively, as determined by comparing the electrophoretic mobility of each band with that of five protein standards of known molecular weights. Whereas by ID only a single precipitin line was obtained when each DES-Ag was reacted against either the homologous or heterologous rabbit antiserum, two separate precipitin lines were evident by IE. Although the DES-Ag appeared as a single line in the gel diffusion studies, it is composed of two distinct antigenic components that differ with respect to molecular weight and electrophoretic mobility.

The chemical composition of the DES-Ag of *T. denticola* is very similar to the chemical composition of the outer envelope of other members of the order *Spirochaetales*. Wachter and Johnson (23) reported that the outer sheath of *T. phagedenis* biotype Reiter is composed of proteins (65%), hexoses (2%), and fatty acids, including oleic and palmitic acids. Trace amounts of *N*-acetylglucosamine (0.3%) were also detected. Ziegler and Van Eseltine (24) studied the chemical composition of the outer envelope of *Leptospira pomona* and reported that it is composed of proteins (47%), carbohydrates (27%), and lipids (23%). Fatty acids, including palmitic, oleic, and stearic acids, were identified by gas-liquid chromatography. The similarities between the chemical composition of the DES-Ag of *T. denticola* and the outer envelope of other spirochetes are an indication that these antigens may be components of the treponemal outer sheath.

Additional evidence indicating that DES-Ag is located near the cell surface was obtained by both MA and ID studies with hyperimmune rabbit antiserum to the DES-Ag of serotype TT. When this antiserum was reacted against whole cells of the homologous serotype by MA, the titer was 1:640. The fact that antiserum prepared specifically against DES-Ag had the ability to agglutinate whole cells is a further indication that DES-Ag is a surface antigen. When this antiserum was adsorbed with whole cells of serotype TT and then reacted against the DES-Ag of the homologous serotype by ID, no precipitin lines were observed. Adsorption studies were also performed with glutaraldehyde-fixed cells of *T. denticola* to eliminate the possibility of nonspecific lysis of the treponemal cells during the adsorption process. Adsorption of specif-

ic anti-DES-Ag serum with glutaraldehyde-fixed cells also resulted in the total elimination of precipitating antibodies against the DES-Ag of serotype TT by ID. It may be concluded from the results of both the chemical analysis studies and the adsorption studies that the DES-Ag of *T. denticola* is a component of the outer envelope of the treponemal cell. Similar adsorption studies carried out by Berman et al. (2) have shown that a soluble antigen extracted from *Brucella abortus* was located near the cell surface.

## ACKNOWLEDGMENTS

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