Treponema brennaborense sp. nov., a novel spirochaete isolated from a dairy cow suffering from digital dermatitis

Kirstin Schrank,1 Bong-Kyu Choi,2 Siegfried Grund,3 Annette Moter,4 Klaus Heuner,4 Herbert Nattermann1 and Ulf B. Göbel4

Author for correspondence: Ulf B. Göbel. Tel: +49 30 2093 4715. Fax: +49 30 2093 4703. e-mail: ulf.goebel@charite.de

INTRODUCTION

Digital dermatitis (DD) is an acute or chronic inflammatory disease of the bovine foot and was first described by Cheli & Mortellaro (1974). The infection occurs with high incidence and leads to a significant decrease in body weight and milk production, causing significant economic losses worldwide (Blowey & Sharp, 1988). The painful DD lesions, typically found along the coronary band, are red, ulcerative and have a characteristic foul odour (Blowey & Sharp, 1988). Due to a loss of keratin, the superficial layers of the epidermis are eroded, leading to epithelial hyperplasia and sometimes to a granulomatous surface similar to a strawberry.

Gram-negative rods and significant numbers of spirochaetes were found by microscopic analyses in touch-preparations of specimens taken from DD lesions (Choi et al., 1997; Done et al., 1993; Döpfer et al., 1997; Read et al., 1992). Porphyromonas levii, Fusobacterium necrophorum, Fusobacterium nucleatum, Prevotella oralis, Prevotella denticola, Prevotella bivia (Nattermann et al., 1996) and as yet unclassified spirochaetes (Walker et al., 1995) were isolated from DD specimens. Recently, we identified by electron microscopy different spirochaetal morphotypes in DD lesions (Grund et al., 1995). The presence of high numbers of spirochaetes suggested a causative role of these bacteria in the aetiopathogenesis of this infection (Collighan & Woodward, 1997; Döpfer et al., 1997; Read et al., 1992; Walker et al., 1997). However, the aetiology of this mixed bacterial infection is still under discussion.

Previous molecular analyses of DD material revealed five spirochaetal phylotypes all clustering within the genus Treponema (Choi et al., 1997), some of which were closely related to treponemes associated with human periodontitis (Choi et al., 1997; Collighan & Woodward, 1997; Rijpkema et al., 1997).

Here we describe a novel strain isolated from DD lesions in cattle. Phenotypic and genotypic charac-

**Abbreviations**: DD, digital dermatitis; FISH, fluorescence in situ hybridization.

The GenBank/EMBL accession number for the 16S rDNA sequence of the novel isolate Treponema brennaborense is Y16568.
terization indicates that this strain represents a novel treponeme species. In this paper we analysed its protein pattern and morphological and biochemical characteristics.

**METHODS**


**Material, media and culture conditions.** A biopsy (1 × 2 × 0.5 cm) from an infected dairy cow (ear-clip: 1219, Dretzener Landprodukte, Land Brandenburg) was taken from the plantar site of a bulbus that showed the typical clinical appearance of DD and was transported to the laboratory immediately. It was cut into pieces of 0.5 × 0.3 × 0.3 cm and incubated in 5 ml OMIZ (Oral Microbiologie und Immunologie Zürich)-Pat medium for 1 hour at 37 °C. This chemically defined liquid culture medium, developed by Wyss (1992), supports growth of a variety of *Treponema* strains (Wyss et al., 1996).

After incubation, aliquots (100 µl) were mixed with 200 µl fresh medium supplemented with antibiotics (rifampin and phosphomycin, 1 and 100 mg l⁻¹, respectively), transferred into 96-well microtiter plates and subjected to a limit dilution procedure (Leschine & Canale-Parola, 1980; Wyss, 1992). After 14 days incubation at 37 °C in GasPak anaerobic jars (Landprodukte, Land Brandenburg) was taken from the wound of the bulbus and incubated in 5 ml OMIZ-Pat medium for 1 hour at 37 °C. This chemically defined liquid culture medium, developed by Wyss (1992), supports growth of a variety of *Treponema* strains (Wyss et al., 1996).

After incubation, aliquots (100 µl) were mixed with 200 µl fresh medium supplemented with antibiotics (rifampin and phosphomycin, 1 and 100 mg l⁻¹, respectively), transferred into 96-well microtiter plates and subjected to a limit dilution procedure (Leschine & Canale-Parola, 1980; Wyss, 1992). After 14 days incubation at 37 °C in GasPak anaerobic jars (AnaeroGen), the samples were examined by dark-field microscopy (Olympus BH2-RFCA; Carl-Zeiss) to determine growth and cell morphology.

Aliquots from wells containing spirochaetes were plated onto OMIZ-Pat agar (1:5%, w/v) plates. Within 5–10 days, diffuse colonies were observed within the agarose. Single colonies were streaked out onto fresh agar plates. To obtain pure cultures, the procedure was repeated three times. Afterwards the strain was removed, transferred to liquid OMIZ-Pat medium and incubated anaerobically until the late-exponential growth phase (6 × 10⁸ bacteria ml⁻¹) was reached. Bacteria were stored at −80 °C in liquid growth medium supplemented with 15% (v/v) glycerol.

**Electron microscopy.** For scanning electron microscopy, colony material (1 × 1 mm) was fixed according to the OTOTO method (Kelley et al., 1973), dried with Peldri II (Ted Pella) and sputter-coated with gold–palladium (5 nm) (SC 501; Emscope).

For transmission electron microscopy, bacteria grown in liquid culture or material of one colony were directly applied to collodion-coated copper grids. After sedimentation of the bacteria and removal of remaining fluid, the samples were directly negatively stained by the method of Nermut (1973), or after fixation in a moist chamber with glutaraldehyde and 2% (v/v) phosphotungstic acid for 1–5 min. The grids were washed with PBS and double-distilled water several times and then air-dried. The samples were examined with a Phillips EM 400 electron microscope.

**Enzyme activities.** For enzyme activity testing, 20 ml bacteria grown in OMIZ-Pat was centrifuged (1305 g, 10 min). To determine catalase activity, cell pellets were covered with 3% (v/v) H2O2 and observed for 15 min for bubble evolution. For API ZYM systems and Rapid ID 32A systems (bioMérieux) the cells were resuspended in double-distilled water and examined according to the manufacturer’s recommendations.

**SDS-gel electrophoresis.** SDS-PAGE was performed by the method of Laemmli (1970). For protein pattern analysis all strains were grown in OMIZ-Pat at 37 °C. Bacteria were harvested by centrifugation (1305 g, 10 min). The pellet was resuspended in 100 µl lysis buffer (500 mM Tris/HCl, pH 9.0; 20 mM EDTA; 10 mM NaCl; 1%, w/v, SDS). The protein concentration of the lysate was determined by the method of Lowry (DC Protein Assay; Bio-Rad). An aliquot containing 10 µg whole-cell protein was taken into two volumes of loading buffer, incubated for 5 min at 95 °C and loaded onto an SDS-polyacrylamide (10%, v/v) gel. After electrophoresis the gel was stained with Coomassie brilliant blue R-250.

**DNA isolation.** For isolation of genomic DNA, 8 ml treponeme culture grown to exponential phase in OMIZ-Pat was pelleted by centrifugation (1305 g, 10 min, 4 °C). The pellet was suspended in 100 µl lysis buffer containing proteinase K (final concentration of 200 µg ml⁻¹; Boehringer Mannheim) and incubated for 1 hour at 52 °C. Phenol/chloroform extraction and all further steps have been described previously (Choi et al., 1997). Genomic DNA was resuspended in 50 µl TE buffer (10 mM Tris; 1 mM EDTA) and stored at −20 °C.

**16S rDNA sequencing and phylogenetic analysis.** 16S rDNA was amplified by PCR using universal eubacterial primer TPU1 [5′ AGAGTTTGTGACT(A/C)GGGCTCAG 3′; corresponding to positions 8–27 in *Escherichia coli* 16S rRNA] and biotinylated eubacterial primer RTU8 [5′ AAGAGGTGATCC(T/G)CC(G/A)CA 3′; corresponding to positions 1541–1522 in *E. coli* 16S rRNA] as described previously (Choi et al., 1994). For sequence determination of the reverse strand, primer RTU8 and biotinylated primer TPU1 were used to amplify the appropriate almost full-length 16S rDNA fragment. Biotinylated strands were separated by using streptavidin-coated paramagnetic beads (Dynabeads; Dynal Hamburg). Infrared labelled universal eubacterial primers were used as sequencing primers (Lane et al., 1985; Weisburg et al., 1991). A Thermo Sequenase fluorescence-labelled primer cycle sequencing kit (Amersham) and a model 4000 LI-COR automated sequencer (MWG-Biotec) were used according to the manufacturers’ instructions.

The 16S rDNA sequence of the new isolate was compared with those of all currently available sequences from the public databases (EMBL, GenBank) using the sequence analysis program HCAP 4.0 (Deutsches Krebsforschungszentrum, Heidelberg, Germany). For phylogenetic analysis the TRECON version 3.1 software package was used (van de Peer & De Wachter, 1993). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987). Multiple base changes at single positions were corrected by the method of Jukes & Cantor (1969). All positions were included in the distance calculation.

**Phyotype-specific rDNA-based oligonucleotide probe.** Specific oligonucleotide probe DDK5/3 (5′ CCTCAACAGCTCCTAACTCCTC 3′; corresponding to positions 184–204 in *E. coli* 16S rRNA) was designed. The specificity of probe DDK5/3 was assessed by dot-blot hybridization using PCR-amplified 16S rDNA from 34 related and unrelated bacteria.
Treponema brennaborense sp. nov.

**Fig. 1.** (a) Scanning electron micrograph of strain DDS/3T cells grown in liquid medium. Different morphotypes are visible: cells with loose irregular windings and helical and circular forms. Bar, 2 μm. (b) Transmission electron micrograph of a cell of strain DDS/3T with the outer cell membrane visible. The two periplasmic flagella are subterminally attached at each pole. The preparation was negatively stained. Bar, 0.5 μm.

The controls included sequences from uncultured oral treponemes derived from a 16S rDNA library, culturable *Treponema* species and oral pathogens as described by Moter et al. (1998). This probe was used to identify the appropriate organisms by fluorescence in situ hybridization (FISH).

**FISH.** For FISH, bacteria were grown in OMIZ-Pat, pelleted by centrifugation (4000 g, 5 min, 4 °C; Heraeus Labofuge 400) and washed in cold PBS. Fixation and preparation of bacteria were performed as described previously (Gersdorf et al., 1993), except the final methanol and formaldehyde fixation step was omitted. The oligonucleotide DDK5/3 was enzymically labelled at the 3' end with Cy3 (indocarbo-cyanin)-dUTP (Amersham) by using terminal transferase according to the digoxigenin-labelling protocols (Boehringer Mannheim) and hybridization was performed as described previously (Choi et al., 1997). ‘*T. vincentii*’, *T. maltophilia*, ‘*T. phagedenis*’, *T. denticola* CD-1 and *E. coli* served as negative control strains to assess specificity of probe DDK5/3. Control strains were included in every *in situ* hybridization experiment.

The FISH preparations were examined under oil immersion with a ×100 Neofluar objective on an Axioskop epifluorescence microscope (Carl-Zeiss) equipped with a high-pressure mercury bulb (HBO 50, Osram) and narrow-band filter sets HQ-F41-007 and HQ-F41-001 (AHF). Photographs were taken on a Kodak Ektachrome HC 400 film.

**Nucleotide sequence accession numbers.** The EMBL accession numbers for the reference spirochaetal 16S rRNA sequences used in this study are as follows: *Treponema medium*, Y09959; DDKL-13, Y08896; ‘*T. phagedenis*’, M57739; DDKL-4, Y08894; *T. denticola*, M71236; DDKL-3, Y08893; *Treponema pallidum*, M88726; Spirochaeta sp., M71240; *T. maltophilia*, X87140; *T. pectinovorum*, M71237; *Treponema bryantii*, M57737; *Treponema saccharophilum*, M71238; *Treponema sp.*, M59294; *Treponema succinifaciens*, M57738; Spirochaeta aurantia, M57740; Spirochaeta isovaleric, M88720; *Borrelia burgdorferi*, L36-160; *Borrelia anserina*, M72397; *Borrelia hermsii*, M60968; *Brachyspira aalborgi*, Z22781; *Brachyspira* (formerly *Serpu- lina*) *hyodysenteriae*, M57742; *Brachyspira* (formerly *Serpula- lina*) *innocens*, M57744; *Leptotrichia* *illini*, Z21632; *Lepton- spira biflexa*, Z12821; *Leptotrichia* *interrogans*, X17547; isolate 1-9185MED, L78125; isolate 2-1498, L78126; *E. coli*, J01859.

**RESULTS**

**Cultural and morphological characteristics**

Using a chemically defined culture medium, OMIZ-Pat, we isolated by limit dilution a novel small spirochaete. The strain was isolated from a biopsy of a
dairy cow with clinical appearance typical for DD. The temperature for growth of the new isolate was 37 °C in GasPak anaerobic jars. After 5 d incubation the strain formed small white diffuse colonies with a diameter up to 3 mm that penetrated completely into the agar on OMIZ-Pat agar plates.

The cells were 5–8 μm long and 0·25–0·55 μm wide, as shown in Fig. 1. They had a cytoplasmic cylinder surrounded by two periplasmic flagella that originated subterminally at each pole.

In liquid medium the cells exhibited high motility. The cells varied in length depending upon the age of the culture. No growth was seen under aerobic conditions. At a growth temperature of 37 °C the culture reached maximum cell density ($8 \times 10^8$ bacteria ml$^{-1}$; pH 5·5) after 21 h incubation, whereas the cell density at the beginning was $8·5 \times 10^6$ bacteria ml$^{-1}$; pH 6·9. In the stationary phase of liquid culture the cells developed circular forms (Fig. 2b). The addition of 2–10% (v/v) rabbit serum or a growth temperature of 30 °C resulted in a decreased growth rate and the serum has therefore been omitted.

**Protein profile**

The protein profile of the new isolate exhibited major bands in the region between 31 and 66 kDa (Fig. 3).

These were clearly different from those of the reference strains *T. denticola*, *T. pectinovorum*, *T. socranskii* subsp. *buccale*, *T. socranskii* subsp. *paredis*, *T. socranskii* subsp. *socranskii*, *T. vincentii*, *T. maltophilum*, etc.
Treponema brennaborense sp. nov.

**Table 1.** Enzyme activities in treponemes as determined by the API ZYM system

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Strain</th>
<th>Source</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novel isolate</td>
<td>DD5/3T</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Veterinary treponemes</td>
<td>Isolates*</td>
<td></td>
<td>†</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T. maltophilum</td>
<td>1-9185MED</td>
<td></td>
<td>†</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T. pectinovorum</td>
<td>ATCC 33768T</td>
<td>‡</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T. socranskii subsp. buccale</td>
<td>ATCC 35534T</td>
<td>‡</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T. socranskii subsp. paredis</td>
<td>ATCC 35535T</td>
<td>‡</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T. socranskii subsp. socranskii</td>
<td>ATCC 35536T</td>
<td>‡</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>'T. vincentii'</td>
<td>LA-1</td>
<td>‡</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T. amylovorum</td>
<td>Ritz A</td>
<td>‡</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* All published isolates except 1-9185MED.
† Walker et al. (1995).
‡ Wyss et al. (1996).
§ Wyss et al. (1997).

'T. phagedenis' biotype Reiter (Fig. 3). These results demonstrate that the new isolate, designated *Treponema brennaborense*, could be clearly distinguished from the reference species by its protein pattern.

**Enzyme activities**

In Table 1 phenotypic characteristics of strain DD5/3T as detected by the API ZYM system are shown in comparison with the enzyme activities of cultivated oral treponemes, described by Wyss et al. (1996, 1997), and DD treponemes, as published by Walker et al. (1995). The following enzyme activities were found: alkaline phosphatase, C₄ esterase, C₅ esterase lipase, acid phosphatase, naphtholphosphohydrolase, β-galactosidase, α-glucosidase and N-acetyl-β-glucosaminidase. Using the Rapid ID 32A system, the new isolate exhibited the enzyme activities alkaline phosphatase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase and arginine arylamidase and it fermented raffinose and mannose. The catalase test was negative.

**Phylogenetic classification**

The almost complete 16S rRNA sequence (1400 bases) was determined for phylogenetic classification. Comparative 16S rDNA sequence analysis showed highest homology to (cluster 17) group IV oral treponemes according to the phylogenetic tree derived from a periodontitis patient (Choi et al., 1994). The new strain showed sequence similarity of 89.5% to *T. maltophilum*, the as yet only cultured species of group IV (Fig. 4).

The overall levels of sequence similarity between *T. brennaborense* 16S rRNA and those of other cultivable oral treponemes ranged from 83.7 to 89.5% (Table 2).

**FISH**

Among all cultivable treponeme species tested, oligonucleotide probe DDK5/3 specifically detected the novel isolate. FISH analysis showed helical (Fig. 2a) and circular (Fig. 2b) forms of strain DD5/3T.
DISCUSSION

The aetiology of DD, a chronic ulcerative epidermitis of the feet of cattle, is still under discussion. A variety of bacterial species including anaerobic Gram-negative rods have been isolated from DD lesions. However, spirochaetes appear to be the predominant organisms in DD lesions and they have been found to invade the stratum spinosum and dermal papillae (Kimura et al., 1993; Read et al., 1992). Microscopic analysis of touch preparations of infected tissue showed different spirochaetal morphotypes (Choi et al., 1997; Done et al., 1993; Döpfer et al., 1997; Read et al., 1992). A report of a significant humoral response in cattle suffering from DD, as compared to healthy animals without detectable lesions, further suggested a possible role of spirochaetes in the aetiopathogenesis of DD (Walker et al., 1997).

Because of the fastidious nature of spirochaetes only few strains associated with DD in cattle have been cultured so far (Walker et al., 1995). Based upon their enzyme activities and morphological or antigenic characteristics, which were clearly different from those of known spirochaetes, they were classified as members of the genus Treponema (Walker et al., 1995). Recently, we used comparative 16S rDNA analysis to phylogenetically classify DD spirochaetes. All of the spirochaetael sequences of a DD-specific 16S rDNA library clustered within the genus Treponema and were closely related to oral treponemes (Choi et al., 1997).

Here we present data of a novel isolate, derived from a DD lesion of an infected cattle, including biochemical, enzymic, morphological and phylogenetic comparisons to known spirochaetes.

By using culture media developed primarily for the isolation of oral spirochaetes, we successfully cultivated treponemes from DD material. After cloning by limit dilution we obtained an isolate with typical spirochaetal morphology and motility. Electron microscopic analysis showed the presence of a cytoplasmic cylinder and two periplasmic endoflagella, inserting subterminally at each pole. Strain DD5/3°C could be clearly distinguished by its flagellation type from all but one (strain 1-9185MED) of the isolates described by Walker et al. (1995). In addition, all isolates differed by their enzyme activities from our new strain (Table 1). This was also true for strain 1-9185MED, which exhibited trypsin and chymotrypsin activity, but lacked β-galactosidase, α-glucosidase and N-acetyl-β-glucosaminidase activity. None of the oral treponeme species showed an identical enzyme pattern in the API ZYM system (Table 1). Furthermore, the novel isolate showed a different protein pattern compared to all treponemes investigated (Fig. 3).

Comparative 16S rDNA sequence analysis allowed the unambiguous phylogenetic classification of the new isolate as a member of the genus Treponema, different from all strains published by Walker et al. (1995). The data of the similarity matrix constructed from the sequence of strain DD5/3°C and those of 17 other spirochaetes clearly support the species status of the new isolate. As seen in Table 2 our strain exhibited highest homology (89-5%) to T. maltophilum, recently isolated from human subgingival plaque (Wyss et al., 1996).

Multiple sequence alignment allowed us to design an oligonucleotide probe specific for the new strain. The specificity of the probe was tested using FISH on different treponeme cultures. Using FISH and electron microscopic analysis on a T. brennaborense culture, circular morphotypes were detected (Fig. 2b). These forms were primarily found in older cultures of DD isolates (Walker et al., 1995) and other treponeme species (Walker et al., 1995; Wecke et al., 1995; Wolf et al., 1993; Wolf & Wecke, 1994). However, the mechanisms leading to circular forms and their possible biological role are still unknown.
_Treponema brennaborense_ sp. nov.

**Table 2. 16S rRNA sequence similarity matrix for novel treponeme strain DD5/3^T^ and reference organisms**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Phylogenetic group</th>
<th>16S rRNA similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 'T. vincentii'</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>2 DDKL-13^*</td>
<td>I</td>
<td>98:7</td>
</tr>
<tr>
<td>3 T. medium</td>
<td>I</td>
<td>98:8 99:6</td>
</tr>
<tr>
<td>4 'T. phagedenis'</td>
<td></td>
<td>91:0 91:3 91:4</td>
</tr>
<tr>
<td>5 DDKL-4^*</td>
<td>I</td>
<td>90:6 91:2 91:4 99:4</td>
</tr>
<tr>
<td>6 T. denticola</td>
<td>II</td>
<td>90:4 91:6 92:3 93:0</td>
</tr>
<tr>
<td>7 DDKL-3^*</td>
<td>II</td>
<td>89:3 90:2 90:4 92:0 92:1 95:3</td>
</tr>
<tr>
<td>8 T. pallidum</td>
<td></td>
<td>87:6 87:7 87:7 90:4 90:5 89:4 89:1</td>
</tr>
<tr>
<td>9 T. maltophilum</td>
<td>IV</td>
<td>86:6 86:3 86:9 86:4 86:3 87:2 87:1 86:5</td>
</tr>
<tr>
<td>10 Strain DD5/3^T^</td>
<td>IV</td>
<td>87:5 87:5 88:0 85:9 86:3 87:3 86:6 84:2 89:5</td>
</tr>
<tr>
<td>11 T. bryantii</td>
<td></td>
<td>85:9 83:9 84:4 84:8 84:4 85:1 85:1 84:6 83:8 86:7 87:0</td>
</tr>
<tr>
<td>12 T. pectinovorum</td>
<td></td>
<td>84:5 85:1 85:5 84:6 85:1 85:4 84:1 87:9 88:2 87:0</td>
</tr>
<tr>
<td>13 T. amylovorum</td>
<td></td>
<td>82:8 83:2 83:8 84:2 83:7 84:8 84:3 83:0 85:6 86:9 88:8 88:5</td>
</tr>
<tr>
<td>14 T. succinifaciens</td>
<td></td>
<td>79:5 79:8 80:5 80:8 81:5 81:0 80:9 82:6 81:3 83:7 83:5 83:6 85:5</td>
</tr>
<tr>
<td>15 Treponema sp.</td>
<td></td>
<td>82:2 83:4 83:7 83:9 83:5 83:8 83:6 84:7 84:6 85:2 86:6 86:2 88:2</td>
</tr>
<tr>
<td>17 T. socranskii</td>
<td></td>
<td>82:8 82:1 82:8 83:8 83:8 84:2 82:8 82:9 83:9 83:6 84:1 84:9 86:6 83:9 85:1 81:3</td>
</tr>
<tr>
<td>18 Spirichus sp.</td>
<td></td>
<td>87:6 87:6 88:3 86:1 87:5 87:0 87:0 86:5 87:0 85:3 83:9 84:9 82:4 84:9 83:9 83:2</td>
</tr>
</tbody>
</table>

*16S rDNA clone sequences (Choi et al., 1997).*

At present, we have no information about the epidemiology of this species. Interestingly, the new isolate was most closely related to the phylogenetic group IV of oral treponemes but was not found in our DD-specific 16S rDNA library published earlier (Choi et al., 1997), suggesting a higher diversity of DD treponemes as compared to oral treponemes in human periodontitis. Molecular epidemiological analyses using the specific oligonucleotide probe are needed to provide information about the incidence and distribution of _T. brennaborense_ in DD-infected animals. Phenotypic characteristics and 16S rDNA gene sequence data indicate that DD5/3^T^ represents a new species for which we propose the name _Treponema brennaborense._

**Description of Treponema brennaborense sp. nov.**

_Treponema brennaborense_ (bren.na.bo.ren'se. Brennabor, medieval name of Brandenburg, Germany; M. L. neut. adj. brennaborense referring to Brennabor, where the cow was raised from which the organism was first isolated).

Anaerobic, Gram-negative, helically coiled, motile treponeme isolated from a DD biopsy of a dairy cow. Bacterial cells are 5–8 μm long and 2.5–5.5 μm wide. They have a cytoplasmic cylinder and two periplasmic flagella that originate subterminally at each cell pole. In stationary-phase liquid cultures the bacteria develop circular forms. In liquid culture the bacteria exhibit rotational movement. Growth of strain DD5/3^T^ is accompanied by acid production. The optimum growth temperature is 37 °C and maximum cell density of approximately 8 x 10^8^ bacteria ml^-1^ is reached after 21 h incubation. Cells can be stored frozen (−80 °C) in OMIZ-Pat medium supplemented with 15% (v/v) glycerol. Using solid-agarose plates _T. brennaborense_ forms diffuse, submersed white colonies up to 3 mm in diameter within 5 d incubation. Strain DD5/3^T^ ferments raffinose and mannose and exhibits the enzyme activities alkaline phosphatase, _C_₄ esterase, _C_₈ esterase lipase, acid phosphatase, naphtholphosphohydrolase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase and arginine arylamidase, as determined by the API ZYM and Rapid ID 32A systems. Catalase is not produced. The addition of 2–10% (v/v) rabbit serum leads to decreased growth rate. The strain is resistant to rifampin (1 mg l⁻¹) and phosphomycin (100 mg l⁻¹). Strain DD5/3^T^ is designated the type strain and has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) as DSM 12168^T^.

**Distinguishing characteristics.** All previously described treponemes are genetically distinct from _T. brennaborense_ as determined by comparative 16S rDNA sequencing. _T. brennaborense_ could be clearly distinguished by its morphology, protein pattern and enzyme activities from the other cultivable _Treponema_ species. Its flagellation type was different from most of the other cultivable _Treponema_ species. Only _T. denticola, T. pectinovorum_, veterinary isolate 1-9185MED and _T. maltophilum_ exhibited the same flagellation type, but they exhibited different enzyme activities. Furthermore _T. brennaborense_ is distinguishable from _T. maltophilum_ by its lack of α-galactosidase activity and its N-acetyl-β-glucosaminidase activity. _T. brennaborense_ is clearly distinguishable from veterinary isolate 1-9185MED by its lack of trypsin and chymotrypsin activities.
ACKNOWLEDGEMENTS

We thank M. Kachler for excellent technical assistance and J. Gatzmann for the electron microscopy. This study was supported in part by a grant (01KB9318) from the Bundesministerium für Bildung und Forschung to U.B.G.

REFERENCES