

Molecular Typing of Papillomatous Digital Dermatitis-Associated *Treponema* Isolates Based on Analysis of 16S-23S Ribosomal DNA Intergenic Spacer Regions

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Papillomatous digital dermatitis (PDD), an emerging infectious disease of cattle, is characterized by painful, ulcerative foot lesions. The detection of high numbers of invasive spirochetes in PDD lesions suggests an important role for these organisms in the pathogenesis of PDD. PDD-associated spirochetes have phenotypic characteristics consistent with members of the genus *Treponema*. Partial 16S ribosomal DNA (rDNA) sequence analysis of clonal isolates from California cattle showed that they comprise three phylotypes which cluster closely with human-associated *Treponema* spp. of the oral cavity (*T. denticola* and *T. medium/T. vincentii*) or genital area (*T. phagedenis*). The goal of our study was to apply 16S-23S rDNA intergenic spacer region (ISR) sequence analysis to the molecular typing of U.S. PDD-associated *Treponema* isolates. This methodology has potentially greater discriminatory power for differentiation of closely related bacteria than 16S rDNA analysis. We PCR amplified, cloned, and sequenced the ISRs from six California PDD-associated *Treponema* isolates and, for comparative purposes, one strain each of *T. denticola*, *T. medium*, *T. vincentii*, and *T. phagedenis*. Two ISRs that varied in length and composition were present in all the PDD-associated *Treponema* isolates and in *T. denticola*, *T. medium*, and *T. phagedenis*. ISR1 contained a tRNA^{Ala} gene, while ISR2 contained a tRNA^{Ile} gene. Only a single ISR (ISR1) was identified in *T. vincentii*. Comparative analyses of the ISR1 and ISR2 sequences indicated that the California PDD-associated *Treponema* isolates comprised three phylotypes, in agreement with the results of 16S rDNA analysis. PCR amplification of the 16S-tRNA^{Ile} region of ISR2 permitted rapid phylotyping of California and Iowa PDD-associated *Treponema* isolates based on product length polymorphisms.

Papillomatous digital dermatitis (PDD), also referred to as digital dermatitis or hairy footwart, was first described in cattle in Italy in 1974 (2). In 1980, Rebhun et al. (13) reported the presence of PDD in dairy cattle in New York. PDD is now found throughout most of the United States. Analysis of data from the National Animal Health Monitoring System Dairy 1996 study indicated that PDD was reported in cattle from 43.5% of U.S. dairy herds (20). In 78% of the affected herds, the first PDD cases occurred in 1993 or later.

PDD typically presents in dairy cattle as lameness episodes of variable severity (10, 20). It is an acute or chronic ulcerative condition that affects the skin on the bulbs of the heel or the interdigital cleft. PDD is characterized by erosion of the superficial layers of the epidermis, epithelial hyperplasia and hypertrophy, pain, swelling, and a foul odor. Lesions usually occur on the hind feet and are prone to bleeding. Early lesions are circumscribed with a red, granular (strawberry-like) appearance and variable degrees of proliferation of filiform papillae. Mature lesions are more proliferative and may have long wart-like projections. If untreated, PDD can persist for months. Significant economic losses associated with PDD oc-

cur due to reduced milk production, impaired fertility, premature culling, and the costs of treatment and control efforts (20).

The response of PDD lesions to antibiotics is strongly suggestive of a bacterial etiology. However, the nature and location of the PDD lesions, coupled with the presence of contaminating fecal and environmental bacteria, have made definitive identification of the etiologic agent(s) difficult. Interestingly, large numbers of spirochetes are consistently found in superficial PDD lesions and also in deeper tissues where other bacteria are rarely observed (3–6, 9, 12, 14). The role of these spirochetes in the pathogenesis of PDD is currently unknown. In 1995, Walker et al. (19) reported the first in vitro cultivation of PDD-associated spirochetes from California dairy cattle. The fastidious, anaerobic spirochetes were differentiated into two phenotypes based on morphological, antigenic, and enzymatic properties. Both phenotypes have characteristics that are consistent with spirochetes in the genus *Treponema*. Read and Walker (11) showed by immunohistochemistry that organisms similar to the California PDD-associated *Treponema* isolates were present in PDD lesion biopsies obtained from cattle in 16 countries. Spirochetes were absent in cattle with no history of PDD.

Comparative 16S ribosomal DNA (rDNA) sequence analysis of several clonal isolates of California PDD-associated *Treponema* isolates indicated that they represent three phylotypes that cluster most closely with the human-associated

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Treponema spp. found in the oral cavity (*T. denticola* and *T. medium*/*T. vincentii*) or the genital area (*T. phagedenis*) (R. L. Walker, D. H. Read, S. J. Sawyer, and K. J. Loretz, Abstr. 79th Annu. Meet. Conf. Res. Workers Anim. Dis., abstr. 17, 1998). Using 16S rDNA sequence analysis, Choi et al. (3) identified five phylotypes of PDD-associated *Treponema* in pooled punch biopsies of lesions from German cows. Three of the phylotypes clustered with *T. denticola*, *T. vincentii*, or *T. phagedenis*. The remaining two phylotypes clustered with the group IV oral treponemes that are also associated with periodontitis. Moter et al. (9) showed differential distribution of the German PDD-associated *Treponema* phylotypes in lesions, suggesting that the development of deep lesions may correlate with the presence of a particular phylotype or combination of phylotypes.

We hypothesized that analysis of the 16S-23S rDNA intergenic spacer regions (ISRs) of the PDD-associated *Treponema* isolates would enhance the discriminatory capability of 16S rDNA analysis due to potential heterogeneity in the length and composition of the ISRs (8, 16). We present here a comparative sequence analysis of the 16S-23S rDNA ISR1 and ISR2 of six California PDD-associated *Treponema* isolates. Additionally, we show that PDD-associated *Treponema* isolates from California and Iowa cattle can be rapidly phylotyped based on PCR product length polymorphisms of the 16S-tRNA^{Ile} region of ISR2.

MATERIALS AND METHODS

Bacterial strains and cultivation. Eight clonal isolates of PDD-associated *Treponema* isolates (1-9185-MED, 2-1498, 7-2009, 9-227, 9-3143, 9-3301, 9-3379, and 9-3528), originally obtained from California dairy cows, were grown anaerobically (BBL GasPak Plus; Becton Dickinson, Cockeysville, Md.) at 37°C in oral treponeme enrichment (OTE) broth (Anaerobe Systems, Morgan Hill, Calif.) (19). The first number for each isolate indicates the dairy farm, and the second number indicates the cow from which the isolate was obtained. *T. denticola* ATCC 35405 (American Type Culture Collection, Manassas, Va.) and *T. phagedenis* biovar Reiter were grown anaerobically as described previously (18). *T. vincentii* ATCC 35580 (American Type Culture Collection) was grown anaerobically in Spirolate broth (Becton Dickinson) supplemented with 0.05% (NH₄)₂SO₄, 10% heat-inactivated normal rabbit serum, and 0.001% thiamine pyrophosphate. *T. medium* G7201 was grown anaerobically in NOS medium (15). Spirochete cell numbers were quantitated by dark-field microscopy. *Escherichia coli* DH5α (Life Technologies, Rockville, Md.), which was used as the host strain for preparation of plasmid DNA for DNA sequence determinations, was grown at 37°C in Luria-Bertani (LB) broth or on LB agar containing 100 μg of ampicillin/ml.

Genomic DNA isolation, PCR amplification, and cloning of the *Treponema* 16S-23S rDNA ISRs. Genomic DNA was isolated from $\approx 2 \times 10^9$ to 4×10^9 cells of the California PDD-associated *Treponema* isolates and the human-associated *Treponema* spp. with the Wizard genomic DNA purification kit (Promega, Madison, Wis.). The 16S-23S rDNA ISRs were PCR amplified (Expand Long Template PCR system; Roche Diagnostics Corp., Indianapolis, Ind.) with 10 ng of genomic DNA as the template with (i) the forward (5'-TTGTACACACCGCC CGTCA-3') and reverse (5'-GGTACCTTAGATGTTTCAGTTC-3') primer set for *T. vincentii* and the California PDD-associated *Treponema* isolates 1-9185MED, 2-1498, 9-3143, and 9-3528 or (ii) the forward (5'-CACACCGCC CGTCA-3') and reverse (5'-CTATCTTCGCTTGACC-3') primer set for *T. denticola*, *T. medium*, *T. phagedenis*, and the California PDD-associated *Treponema* isolates 7-2009 and 9-3379.

To prevent spurious PCR products, the water that was used for PCR was treated with DNase I (Life Technologies) per the manufacturer's suggested protocol. PCR products were electrophoresed on a 0.8% agarose gel and stained with ethidium bromide. A DNA fragment of the anticipated size was eluted, purified, and cloned into the plasmid vector pGem-T or pGem-T Easy (Promega). Recombinant plasmids were transformed into *E. coli* DH5α, and the transformants were identified by the standard blue-white screening procedure (Promega). Recombinant plasmids from selected clones were purified with the

Wizard Miniplus DNA purification system (Promega), and the inserts were confirmed by agarose gel electrophoresis following restriction enzyme digestion.

DNA sequencing and analysis. The nucleotide sequences of the *Treponema* 16S-23S rDNA ISRs were determined with vector-based M13 primers at the University of North Carolina at Chapel Hill Automated DNA Sequencing Facility. Both DNA strands of the inserts from four to six recombinant plasmids for each cloned, PCR-amplified ISR product were sequenced for accuracy. DNA sequence analyses were performed with MacVector 7.0 (Accelrys Inc., Madison, Wis.) and the Wisconsin Genetics Computer Group software package, version 10 (University of Wisconsin Biotechnology Center, Madison, Wis.). Multiple sequence alignments were constructed with PileUp and adjusted manually. Phylogenetic analyses were performed with PAUPSearch (Genetics Computer Group). Unrooted trees were generated with maximum parsimony with a heuristic search setting with stepwise addition and tree-bisection-reconnection branch swapping. Bootstrap analysis was performed with 10,000 resamplings.

PCR amplification of the 16S-tRNA^{Ile} region of ISR2. Genomic DNA of the California PDD-associated *Treponema* isolates was prepared as described above. Genomic DNA from four Iowa PDD-associated *Treponema* isolates (1A, 3A, 4A, and 5B), originally isolated by D. Trott, T. Stanton, and M. Wannemuehler, was provided by R. Zuerner (National Animal Disease Control Center, Ames, Iowa). Approximately 10 ng of genomic DNA of each PDD-associated *Treponema* isolate was subjected to PCR amplification with the forward (5'-CCGCCCCGTC ACACCATCC-3') and reverse (5'-CCCCTTCCTTATCAGAGA-3') primers. These sequences are complementary to conserved regions in the 16S rRNA gene and tRNA^{Ile} gene, respectively. The amplification program consisted of a 94°C hold for 2 min and then denaturation at 94°C for 30s, annealing at 52.5°C for 30 s, and extension at 72°C for 1 min for 30 cycles, with an additional extension at 72°C for 3 min. The PCR products were electrophoresed on a 1.7% agarose gel, stained with ethidium bromide, and photographed.

Nucleotide sequence accession numbers. The nucleotide sequences of the *Treponema* 16S-23S rDNA ISRs were deposited in GenBank under accession numbers AF179251 to AF179266, AF209195, AF378325, and AF378326.

RESULTS

Comparison of 16S-23S rDNA ISRs of PDD-associated *Treponema* isolates and related human-associated *Treponema* spp. PCR amplification of the 16S-23S rDNA ISRs of six California PDD-associated *Treponema* isolates and four human-associated *Treponema* spp. yielded one predominant product for each of the organisms. Cloning and sequencing of the individual PCR products revealed the presence of two ISRs (ISR1 and ISR2) for each *Treponema* isolate or species except *T. vincentii*, for which only one ISR (ISR1) was identified. ISR1 contains a centrally located tRNA^{Ala} gene, whereas ISR2 contains a centrally located tRNA^{Ile} gene (Fig. 1 and 2). Both tRNA genes are 75 bp in length, and each tRNA gene is virtually identical among the *Treponema* isolates and species included in this study. In contrast, the regions immediately 5' and 3' of the tRNA genes (i.e., following the 3' end of 16S rDNA and preceding the 5' end of 23S rDNA) are variable in nucleotide sequence length and composition between groups of PDD-associated *Treponema* isolates and among the four human-associated *Treponema* spp. (Fig. 1 and 2). A pairwise comparison of the nucleotide sequence similarity of ISR1 and ISR2 indicated that the six California PDD-associated *Treponema* isolates comprised three groups: (i) 1-9185MED, 9-3143, and 9-3528; (ii) 9-3379; and (iii) 2-1498 and 7-2009 (Table 1). The group 2 and group 3 PDD-associated *Treponema* isolates were more closely related to *T. medium*/*T. vincentii* and *T. phagedenis*, respectively, than they were to the group 1 PDD-associated *Treponema* isolates. Additionally, two (9-3143 and 9-3528) of the three group 1 isolates had identical ISR1 and ISR2 sequences. These isolates, which were obtained from separate dairy cows on the same farm, may be the same organism.

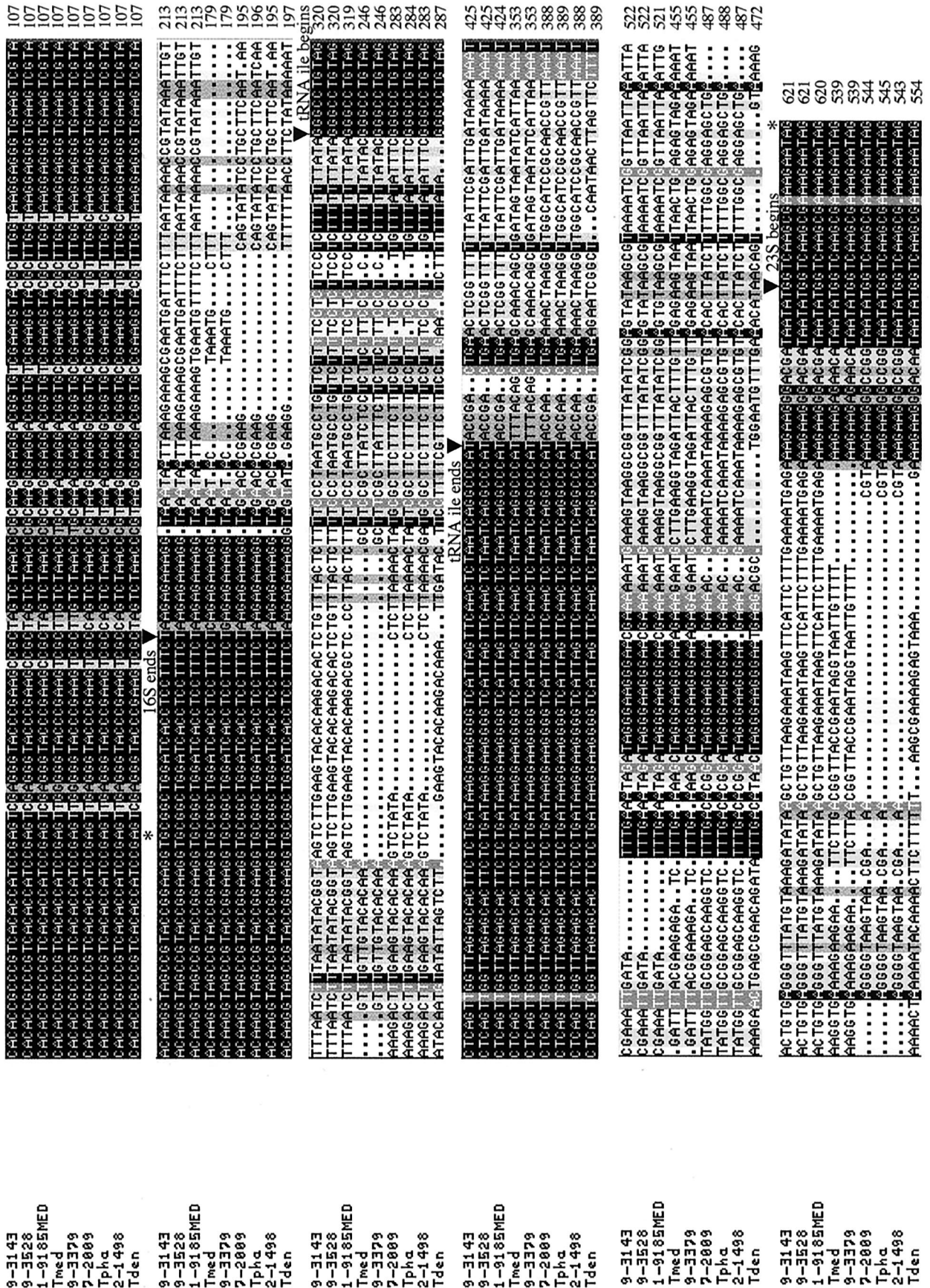


FIG. 2. Multiple sequence alignment of 16S-23S rDNA ISR2 of the six California PDD-associated *Treponema* isolates and the related human-associated *Treponema* spp. The 3' end of the 16S rDNA, the tRNA^{leu} gene, and the 5' end of the 23S rDNA are indicated. Abbreviations: Tmed, *T. medium*; Tpha, *T. phagedenis*; Tden, *T. denticola*.

TABLE 1. ISR1 and ISR2 nucleotide sequence similarity values for the six California PDD-associated *Treponema* isolates and the related human-associated *Treponema* spp.^a

Isolate or species	% Similarity to:								
	1-9185MED	9-3143	9-3528	9-3379	2-1498	7-2009	<i>T. denticola</i>	<i>T. medium</i>	<i>T. phagedenis</i>
PDD 1-9185MED		98.4	98.4	60.5	62.8	57.8	65.3	60.5	60.3
PDD 9-3143	98.2		100.0	60.0	54.2	54.5	63.8	60.0	56.2
PDD 9-3528	98.2	100.0		60.0	54.2	54.5	63.8	60.0	56.2
PDD 9-3379	63.9	61.0	61.0		57.7	57.7	64.8	97.8	58.0
PDD 2-1498	62.3	62.6	62.6	57.3		99.7	58.6	57.4	99.5
PDD 7-2009	62.3	62.6	62.6	57.3	100.0		60.0	57.4	99.7
<i>T. denticola</i>	61.5	62.0	62.0	54.8	57.2	57.2		65.7	59.8
<i>T. medium</i>	59.1	59.4	59.4	93.7	57.4	57.4	54.0		57.1
<i>T. phagedenis</i>	56.2	58.0	58.0	56.0	99.2	99.2	58.4	55.5	
<i>T. vincentii</i>	54.0	55.7	55.7	77.4	59.3	59.3	54.8	76.4	58.9

^a ISR1 similarity is on the lower left. ISR2 similarity is on the upper right. An ISR2 was not identified in *T. vincentii*.

Comparison of 16S-23S rDNA ISRs of California PDD-associated *Treponema* isolates and other spirochetes.

Although the lengths of the 16S-23S rDNA ISRs differ, the number of ISRs and the tRNA gene content of the individual ISRs were identical for the six PDD-associated *Treponema* isolates and for most of the human-associated *Treponema* spp., including *T. pallidum* (syphilis agent) and *T. pertenuis* (yaws agent) (1) (Table 2). In contrast to the *Treponema* spp., *Borrelia burgdorferi* and *Borrelia hermsii* contain one large 16S-23S rDNA ISR in which both tRNA^{Ala} and tRNA^{Ile} genes are present (7; C. Ojaimi and B. E. Davidson, Abstr. 99th Annu. Meet. Am. Soc. Microbiol., abstr. D/B-256, 1999). *Brachyspira hyodysenteriae* (22) and the saprophytic and pathogenic *Leptospira* spp. do not contain linked 16S-23S rDNA genes and thus lack ISRs (21). *Leptonema illini* contains two 16S-23S rDNA ISRs. Only one of

the ISRs has been sequenced, and it does not contain a tRNA gene (21).

ISR-based phylogeny of California PDD-associated *Treponema* isolates. Phylogenetic analyses were performed with the ISR1 and ISR2 nucleotide sequences of the six California PDD-associated *Treponema* isolates and the four related human-associated *Treponema* spp. with the maximum-parsimony method. The tree that was generated from the ISR1 data showed that the California PDD-associated *Treponema* isolates formed three distinct clusters (Fig. 3). These clusters, designated phylotypes 1, 2, and 3, correspond to the three groups identified by pairwise comparisons of the ISR data (Table 1). Bootstrapping confirmed the robustness of the phylogeny assignments. Phylotype 1 isolates 1-9185MED, 9-3143, and 9-3528 were less closely related to the human-associated *Treponema* spp. than were the phylotype 2 and phylotype 3 isolates. Phylotype 2 isolate 9-3379 grouped with *T. medium* and *T. vincentii*. Phylotype 3 isolates 2-1498 and 7-2009 grouped with *T. phagedenis*. Interestingly, none of the phylotypes was closely related to *T. denticola*. The tree that was generated from the ISR2 data was essentially identical to the ISR1 tree (data not shown). The clustering of the California PDD-associated *Treponema* isolates into three phylotypes is consistent with the results of phylogenetic analysis performed with 16S rDNA sequence data (R. L. Walker, D. H. Read, S. J. Sawyer, and K. J. Loretz, Abstr. 79th Annu. Meet. Conf. Res. Workers Anim. Dis., abstr. 17, 1998).

Typing of PDD-associated *Treponema* isolates based on length polymorphisms of PCR-amplified 16S-tRNA^{Ile} region of ISR2. Availability of the 16S-23S rDNA ISR sequences of the California PDD-associated *Treponema* isolates facilitated the design of primer sets for PCR amplification of the 16S-tRNA^{Ala} and 16S-tRNA^{Ile} regions of ISR1 and ISR2, respectively. PCR amplification of the 16S-tRNA^{Ile} region of the six California PDD-associated *Treponema* isolates yielded a single, reproducible PCR product that was more readily interpretable based on intergel comparisons than the product of the 16S-tRNA^{Ala} region. Isolates of the same phylotype yielded products of virtually identical size (i.e., phylotype 1, ~330 bp; phylotype 2, ~260 bp; and phylotype 3, ~300 bp) (Fig. 4).

We PCR amplified the 16S-tRNA^{Ile} region of two additional California PDD-associated *Treponema* isolates (9-227 and

TABLE 2. Comparison of 16S-23S rDNA ISRs of six California PDD-associated *Treponema* isolates and other spirochetes

Spirochete	Copy no. of 16S-23S rRNA genes ^a	ISR length (nucleotides)	tRNA gene(s) in ISR
PDD 1-9185MED	2	452/449	Ala or Ile
PDD 9-3143	2	454/450	Ala or Ile
PDD 9-3528	2	454/450	Ala or Ile
PDD 9-3379	2	367/368	Ala or Ile
PDD 2-1498	2	380/373	Ala or Ile
PDD 7-2009	2	380/373	Ala or Ile
<i>T. denticola</i>	2	400/383	Ala or Ile
<i>T. medium</i>	2	365/369	Ala or Ile
<i>T. vincentii</i>	1	405	Ala
<i>T. phagedenis</i>	2	384/374	Ala or Ile
<i>T. pallidum</i>	2	302/292	Ala or Ile
<i>B. burgdorferi</i>	1 ^b	3051	Ala and Ile
<i>B. hermsii</i>	1 ^c	5854	Ala and Ile
<i>Leptonema illini</i>	2	435/ND ^d	None/ND
<i>Leptospira biflexa</i>	None	NA ^e	NA
<i>Leptospira interrogans</i>	None	NA	NA
<i>Brachyspira hyodysenteriae</i>	None	NA	NA

^a Copy number of linked 16S-23S rRNA genes based on cloning, Southern hybridization, and/or genomic sequence data.

^b Unusual arrangement (16S-ISR-23S-5S-23S-5S). ISR contains a tRNA^{Ala} gene, a tRNA^{Ile} gene, and two open reading frames.

^c ISR contains a tRNA^{Ala} gene, a tRNA^{Ile} gene, and 5 open reading frames.

^d ND, not determined.

^e NA, not applicable.

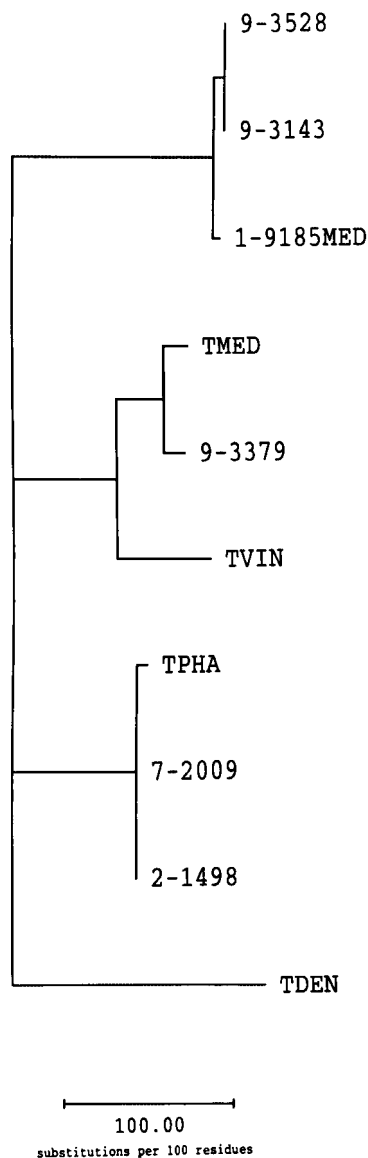


FIG. 3. Unrooted phylogenetic tree (phylogram) inferred from the 16S-23S rDNA ISR1 sequences (within the region bracketed by stars [*] in Fig. 1) of the six California PDD-associated *Treponema* isolates and the related human-associated *Treponema* spp. Bootstrap analysis (10,000 resamplings) supported the placement of the California PDD-associated *Treponema* isolates into three phylotypes. Abbreviations: TMED, *T. medium*; TVIN, *T. vincentii*; TPHA, *T. phagedenis*; TDEN, *T. denticola*.

9-3301) and four Iowa PDD-associated *Treponema* isolates (1A, 3A, 4A, and 5B) to test the utility of this approach for phylotyping. Isolates 9-227 and 9-3301 yielded PCR products of ~260 and ~300 bp, respectively, which assigned them to phylotypes 2 and 3, respectively. All four Iowa isolates yielded a PCR product of ~300 bp, which assigned them to phylotype 3. Cloning and sequencing of the PCR products from two Iowa isolates (1A and 4A) indicated that the sequence of their 16S-tRNA^{Ile} region is identical and that it is >99% identical to the 16S-tRNA^{Ile} region of the California phylotype 3 isolates 2-1498 and 7-2009 (data not shown).

The phylotype assignments of the eight California and four

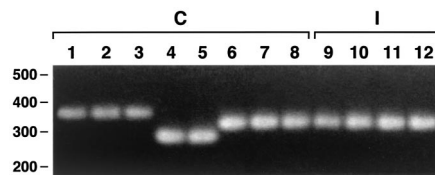


FIG. 4. Agarose gel analysis of PCR amplification products of 16S-tRNA^{Ile} region of eight California (C) and four Iowa (I) PDD-associated *Treponema* isolates. Lanes: 1, 1-9185MED; 2, 9-3143; 3, 9-3528; 4, 9-3379; 5, 9-227; 6, 2-1498; 7, 7-2009; 8, 9-3301; 9, 1A; 10, 3A; 11, 4A; and 12, 5B. The positions of size standards (in base pairs) are indicated on the left side of the gel.

Iowa PDD-associated *Treponema* isolates are in agreement with the results obtained with comparative 16S rDNA sequence analysis (R. L. Walker, D. H. Read, S. J. Sawyer, and K. J. Loretz, Abstr. 79th Annu. Meet. Conf. Res. Workers Anim. Dis., abstr. 17, 1998; D. Trott, personal communication). Our results indicate that clonal isolates of the California and Iowa PDD-associated *Treponema* isolates can be rapidly phylotyped based on the length polymorphisms of their 16S-tRNA^{Ile} region PCR product.

DISCUSSION

Several studies have shown that the 16S-23S rDNA ISR is a useful target for differentiating species or in some cases strains of various bacterial pathogens of humans and animals (8, 16). Our studies represent the first application of this methodology to PDD-associated *Treponema* isolates and to four related human-associated *Treponema* spp. Our results indicated that the PDD-associated *Treponema* isolates have two 16S-23S rDNA ISRs of variable nucleotide length and composition. ISR1 contains a tRNA^{Ala} gene, while ISR2 contains a tRNA^{Ile} gene. The copy number and tRNA gene content of the 16S-23S rDNA ISRs of the PDD-associated *Treponema* isolates are identical to those of the related human-associated *Treponema* spp. with the exception of *T. vincentii*, for which only one ISR was identified.

Based on comparative 16S-23S rDNA ISR sequence analysis, six California PDD-associated *Treponema* isolates were differentiated into three phylotypes. These results are in agreement with those of 16S rDNA sequence analysis (R. L. Walker, D. H. Read, S. J. Sawyer, and K. J. Loretz, Abstr. 79th Annu. Meet. Conf. Res. Workers Anim. Dis., abstr. 17, 1998). However, we observed that 16S-23S rDNA ISR sequence analysis provided somewhat higher discriminatory power than 16S rDNA sequence analysis, since it showed that the California phylotype 1 PDD-associated *Treponema* isolates were not closely related to *T. denticola*.

As noted previously, Choi et al. (3), using comparative 16S rDNA sequence analysis, identified five uncultured phylotypes of PDD-associated *Treponema* that were present in pooled lesion biopsies from four German cows. Three phylotypes (DDKL-3, DDKL-13, and DDKL-4) were most similar to *T. denticola*, *T. vincentii*, or *T. phagedenis*, respectively, and presumably correlate to U.S. phylotypes 1, 2, and 3, respectively. The remaining two phylotypes (DDKL-12 and DDKL-20) had no close relative but clustered to group IV human-associated

oral treponemes. Schrank et al. (17) isolated a novel *Treponema* (*T. brennaborensis*) from a PDD lesion of a German dairy cow. This spirochete, which is genetically distinct from the five phylotypes of Choi et al. (3), is most closely related to *T. maltophilum* (89.5% 16S rDNA similarity). Based on comparative 16S rDNA sequence analysis of PCR products amplified from PDD lesion biopsies, there is no evidence that the DDKL-12 and DDKL-20 phylotypes or *T. brennaborensis* are present in California cattle (R. Walker, unpublished data). Interestingly, only sequences of PDD-associated *Treponema* isolates that are related to *T. denticola* have been PCR amplified from PDD lesions of cattle in the United Kingdom (4, 14).

The availability of the 16S-23S rDNA ISR sequence data from the California PDD-associated *Treponema* isolates prompted us to develop a rapid PCR-based method for the phylotyping of clonal isolates. We found that eight PDD-associated *Treponema* isolates from California cows and four isolates from Iowa cows could be assigned to the correct phylotype based on 16S-tRNA^{Ile} region PCR product length polymorphisms. This method has advantages over 16S rDNA and 16S-23S rDNA ISR sequence analysis because it reproducibly yields a single, readily visible PCR product for each isolate and does not require the expense and time associated with sequencing and analyzing cloned PCR products. Although the total number of isolates that were examined was relatively small, we anticipate that PCR amplification of the 16S-tRNA^{Ile} region will be applicable to additional clonal isolates of PDD-associated *Treponema* isolates. Furthermore, modification of the 16S-tRNA^{Ile} region PCR should allow the development of a PCR-enzyme-linked immunosorbent assay to directly identify PDD-associated *Treponema* phylotypes in lesion biopsies, obviating the need for culture. The results of Moter et al. (9) suggest that the presence of certain PDD-associated *Treponema* phylotypes may correlate with more invasive disease. Thus, the ability to detect and differentiate *Treponema* phylotypes in lesion biopsies would be a useful adjunct to in situ hybridization for studies of the epidemiology and pathogenesis of PDD.

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