JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1991, p. 2774–2778
0095-1137/91/122774-05$02.00/0

Vol. 29, No. 12

Differentiation of *Streptococcus uberis* from *Streptococcus parauberis* by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism Analysis of 16S Ribosomal DNA

B. M. JAYARAO,[1] J. J. E. DORÉ, JR.,[2] G. A. BAUMBACH,[1] K. R. MATTHEWS,[1] AND S. P. OLIVER[1]*

Department of Animal Science, Institute of Agriculture,[1] and Department of Pathobiology, College of Veterinary Medicine,[2] University of Tennessee, Knoxville, Tennessee 37901-1071

Received 5 July 1991/Accepted 19 September 1991

***Streptococcus uberis* type II has been proposed recently as a separate species designated *Streptococcus parauberis* (A. M. Williams and M. D. Collins, J. Appl. Bacteriol. 68:485–490, 1990). Differentiation of *S. parauberis* from *S. uberis* has been possible only by DNA-DNA hybridization or 16S rRNA sequencing, since the biochemical and serological characteristics of the two species are indistinguishable. A simple and reliable technique was developed for differentiating *S. parauberis* (*S. uberis* type II [ATCC 13386]) from *S. uberis* (*S. uberis* type I [ATCC 9927, ATCC 13387, and ATCC 27958]) by restriction fragment length polymorphism (RFLP) analysis of 1.4-kb 16S ribosomal DNA (rDNA). Oligonucleotide primers complementary to 16S rRNA genes were used to amplify 16S ribosomal gene fragments from genomic DNA by polymerase chain reaction. The 1.4-kb 16S rDNA fragment was digested with *Sca*I, *Nsp*I, *Dde*I, and *Ava*II restriction endonucleases. Restriction fragments produced by all four restriction endonucleases were characteristic for each species. RFLP analysis of 16S rDNA from 24 "*S. uberis*" isolates obtained from mammary secretions of dairy cows indicated that all 24 isolates were indeed *S. uberis*.**

Mastitis is the most common disease affecting dairy cows. Mastitis results invariably in decreased productivity and in severe cases results in loss of function of the affected mammary gland and even death. In addition, discarding of milk from affected quarters and treatment of cows with antimicrobial agents results in significant economic losses to dairy producers. The predominant bacteria involved in mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and coliforms (21). Postmilking teat disinfection and use of antimicrobial agents for treatment and prevention of mastitis have reduced the incidence of *S. agalactiae* and *S. aureus* intramammary infections (14, 15). However, the number of cases of mastitis caused by environmental pathogens, *S. uberis* in particular, has increased markedly (2, 5, 11, 13, 19). In many dairy herds, *S. uberis* is responsible for a high proportion of clinical and subclinical mastitis cases in lactating cows and is the predominant organism isolated from mammary glands during the nonlactating period and during early lactation (2, 5, 10, 11, 19).

S. uberis was first described in 1932 (4). Roguinsky (18) compared some physiological properties of strains of *S. uberis* with those of seven other species found in the same habitat and concluded that *S. uberis* was a separate species. Using DNA-DNA hybridization techniques, Garvie and Bramley (6) demonstrated the presence of two *S. uberis* genotypes designated types I and II. In our earlier studies of genomic DNA fingerprinting of *S. uberis*, several DNA fingerprint patterns were observed, which suggested clonal diversity (9). More recently, Williams and Collins (23) determined the nucleotide sequences of 16S rRNA of *S. uberis* types I and II, showed the two genotypes were phylogenetically distinct, and proposed that *S. uberis* type II be designated *Streptococcus parauberis*.

Differentiation of *S. uberis* from *S. parauberis* is difficult because the biochemical and serological characteristics of the two species are essentially indistinguishable. The inability to distinguish *S. uberis* from *S. parauberis* could result in erroneous conclusions concerning the epidemiology of *S. uberis*, such as identification of reservoirs and mode of transmission. In addition, the lack of a suitable method for distinguishing *S. uberis* from *S. parauberis* could affect studies on the pathogenesis of *S. uberis* and hinder development of methods for controlling this important mastitis pathogen. A report by Garvie and Bramley (6) indicated that *S. uberis* type II strains were isolated frequently from British dairy herds. However, no reports on the occurrence of *S. uberis* type II strains in the United States have been published. Thus, a simple and reliable method to differentiate the two organisms is needed; such a method would be of value to research and reference laboratories involved in studies of *S. uberis*.

Sequence comparisons of 16S rRNAs or their genes have provided precise information on molecular and cellular evolution (20). This has led to the determination of phylogenetic and evolutionary relationships among organisms (17). The polymerase chain reaction (PCR) technique can be employed to amplify 16S ribosomal DNA (rDNA) coding regions rapidly (12). In this study, on the basis of the 16S rRNA sequence analysis of *S. uberis* type I and II reported by Williams and Collins (23), two oligonucleotide primers were used, and the region of interest was amplified by PCR. The 16S rDNA fragments amplified from *S. uberis* type I and II strains and from clinical and subclinical isolates were examined by restriction fragment length polymorphism (RFLP) analysis with the objective of differentiating *S. uberis* from *S. parauberis*.

* Corresponding author.

MATERIALS AND METHODS

Bacteria. Four American Type Culture Collection (ATCC; Rockville, Md.) strains, ATCC 9927, ATCC 13387, and ATCC 27958 (*S. uberis* type I) and ATCC 13386 (*S. uberis* type II), were used. In addition, 24 isolates of *S. uberis* obtained from mammary glands of dairy cows with clinical and subclinical mastitis from two University of Tennessee dairy research herds were examined.

All strains were preserved in 10% skim milk and stored at −70°C. The isolates were subcultured from storage media onto 5% blood agar plates and maintained on brain heart infusion agar slants. All strains included in the study were identified to the species level by the Gram-Positive Identification System (Vitek Systems Inc., Hazelwood, Mo.), API Rapid Strep System (Analytab Inc., Plainview, N.Y.), and conventional biochemical tests and serotyped as described previously (8).

Isolation of DNA. Chromosomal DNA of *S. uberis* was isolated by the method described by Hill and Leigh (7) with modifications (9). Cells from 1.5 ml of overnight culture grown in brain heart infusion broth were pelleted by centrifugation (7,000 × *g* for 3 min at 4°C), washed once with 1.0 ml of Tris (10 mM)-EDTA (5 mM), pH 7.8, and resuspended in 325 μl of the same buffer. After addition of 25 μl of mutanolysin (5,000 U/ml; Sigma Chemical Co., St. Louis, Mo.) and 25 μl of freshly prepared lysozyme (10 mg/μl; Sigma), the bacteria were incubated at 37°C for 30 min. Lysis of cells was achieved by addition of 20 μl of sodium dodecyl sulfate (SDS) buffer (20% [wt/vol] SDS in Tris [50 mM]–EDTA [20 mM], pH 7.8) followed by 3 μl of proteinase K (20 mg/ml; Sigma) and further incubation at 37°C for 1 h. Protein was precipitated by addition of 200 μl of saturated NaCl (5 M) followed by agitation for 15 s and was removed by centrifugation (7,000 × *g*) for 10 min at 4°C. The pellet was discarded and the supernatant was subjected to phenol-chloroform (1:1) extraction followed by chloroform-isoamyl alcohol (24:1) extraction. DNA was precipitated from the supernatant with 2.5 volumes of 95% ethanol and 30 μl sodium acetate (1 M) overnight at −20°C. The resulting precipitate was collected by centrifugation at 7,000 × *g* for 5 min at 4°C and then washed in 70% ethanol. The DNA pellet was dried under a vacuum and rehydrated in 30 μl of buffer (Tris [10 mM]–EDTA [1.0 mM], pH 7.5).

DNA primers and PCR amplification. The oligonucleotide primers utilized in this study were synthesized by the Analytical Services Laboratory, University of Tennessee, Knoxville. Sequences corresponded to those within the 16S rRNA gene of *S. uberis* (23). The 5′ primer 5′-CCAAGCTTGCTCAGGACGAACGCT-3′ corresponded to nucleotides 20 through 35, with a *Hin*dIII restriction site (underlined) at the 5′ end. The 3′ primer 5′-CGGGATCCCGCCCGGGAACGTATTCAC-3′ was similar to the broad-range primer p13B described by Chen et al. (3) and corresponded to nucleotides 1374 through 1392 of 16S rRNA (23) with a *Bam*HI restriction site (underlined) incorporated at the 5′ end. Both primers had a theoretical melting temperature of 58°C. The PCR was done in a total volume of 100 μl containing 20 ng of the bacterial DNA preparation, 0.5 μM each primer, 100 μM each deoxynucleoside triphosphate (Boehringer Mannheim, Indianapolis, Ind.), 10 μl of 10× *Taq* buffer, and 2.5 units of *Taq* DNA polymerase (Promega, Madison, Wis.). Each reaction mixture was overlaid with 50 μl of mineral oil and run for 35 cycles in a GTC Thermocycler (Precision Scientific Inc., Chicago, Ill.). One cycle consisted of 90 s at 93°C, 90 s at 56°C, and 90 s at 75°C. On completion, each sample was extracted with chloroform, precipitated with 2 volumes of ethanol, and resuspended in 50 μl of distilled water.

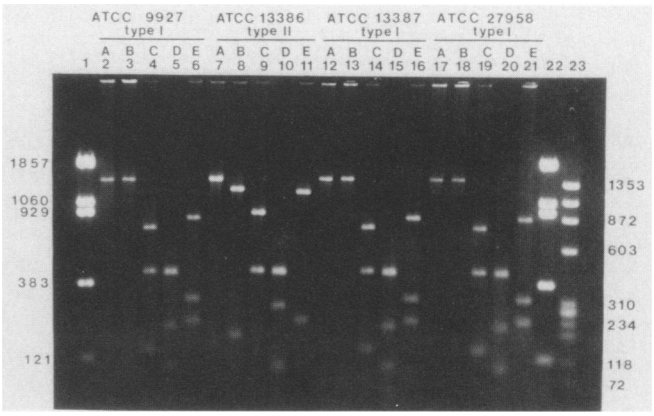

FIG. 1. Restriction endonuclease digestion of 16S rDNA of *S. uberis* ATCC strains followed by electrophoresis on 2% agarose gels. Lanes 1 and 22, *Bst*NI-digested pBR322 DNA; lane 23, *Hae*III-digested φX174 DNA. Different lanes for each ATCC strain: A, uncut 16S rDNA; B, *Sca*I digest; C, *Nsp*HI digest; D, *Dde*I digest; E, *Ava*II digest. Fragment sizes are given in base pairs.

Enzymatic digestion of amplified DNA. DNA (5 μl) was digested for 2 h at 37°C in 20-μl volumes with *Sca*I, *Dde*I, and *Ava*II (all from New England Biolabs, Beverly, Mass.) and *Nsp*HI (Boehringer Mannheim) restriction endonucleases. Determination of the use of suitable restriction endonucleases for RFLP analysis was done by identifying restriction sites on the 16S rDNA sequence with IBI/Pustell sequence analysis programs (16) (Table 1). Digestion of amplified 16S rDNA was repeated to establish reproducibility.

Agarose gel electrophoresis. Restriction endonuclease-digested 16S rDNA fragments were electrophoresed in 2% agarose in Tris-borate-EDTA buffer (0.09 M Tris base, 0.09 M sodium borate, 2.5 mM EDTA, pH 8.3) in gels (20 by 20 cm) at 100 V for 4 h. The gels were stained with ethidium bromide (1.0 μg/ml), and DNA was visualized by UV transillumination (Fotodyne Inc., New Berlin, Wis.) and photographed with type 55 Polaroid film. *Bst*NI-digested pBR322 DNA and *Hae*III-digested φX174 DNA were used as molecular weight markers (New England Biolabs).

RFLP analysis. The negative of the Polaroid film was scanned with a computer-integrated laser densitometer (Ultroscan XL; LKB Produkter AB, Bromma, Sweden). The scans were stored and retrieved for the purpose of comparison and evaluation with the Gelscan XL version 2.0 software package (Pharmacia LKB Biotechnology, Uppsala, Sweden). The number of DNA fragments and their sizes in base pairs were determined.

RESULTS

Amplification of 16S rDNA by PCR. The 16S rDNA fragment was amplified by PCR with genomic DNA of *S. uberis* type I and II strains as the template. The size of the amplified DNA fragment from both type I and II strains was approximately 1,400 bp (Fig. 1 and 2) relative to DNA size markers, as determined by scanning laser densitometric evaluation (Table 1; Fig. 3).

RFLP analysis. The amplified 16S rDNA fragment was

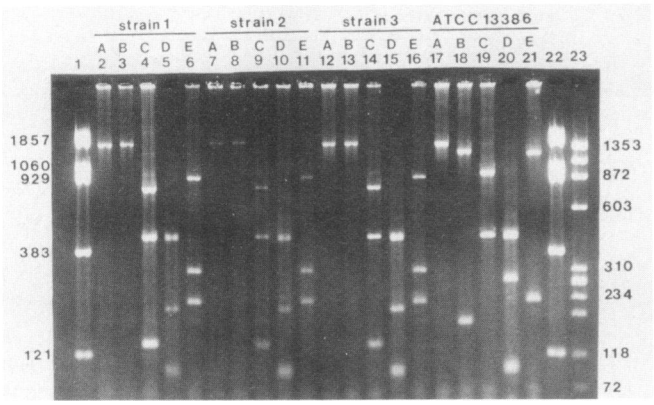

FIG. 2. Restriction endonuclease digestion of 16S rDNA of four β-glucuronidase-negative *S. uberis* strains followed by electrophoresis on 2% agarose gels. Lanes 1 and 22, *Bst*NI-digested pBR322 DNA; lane 23, *Hae*III-digested φX174 DNA. Different lanes for each ATCC strain: A, uncut 16S rDNA; B, *Sca*I digest; C, *Nsp*HI digest; D, *Dde*I digest; E, *Ava*II digest. Fragment sizes are given in base pairs.

digested with four different restriction endonucleases. Restriction endonuclease sites for *Sca*I were absent from *S. uberis* type I, whereas one site corresponding to position 188 was present in *S. uberis* type II. This resulted in two fragments of 170 and 1,230 bp (Table 1; Fig. 3).

Three restriction fragments of 140, 450, and 760 bp were observed in *S. uberis* type I with restriction endonuclease *Nsp*HI. In *S. uberis* type II, two restriction fragments of 450 and 940 bp were observed (Table 1; Fig. 1).

Three fragments of 110, 200, and 450 bp were observed with restriction endonuclease *Dde*I in *S. uberis* type I. Fragments of 110 and 450 bp were observed in *S. uberis* type II. However, a 290-bp fragment was present instead of the 200-bp fragment seen in type I strains. Analysis of band intensities by densitometry indicated that the 450-bp band was a mixture of two different fragments in both type I and type II strains.

Three fragments of 230, 310, and 860 bp were observed in *S. uberis* type I with restriction endonuclease *Ava*II, whereas only two restriction fragments of 230 and 1,170 bp were observed in *S. uberis* type II (Fig. 1 and 2).

Examination of mammary gland isolates of *S. uberis* by RFLP analysis with all four endonucleases revealed that all isolates had RFLP patterns identical to that of the *S. uberis* ATCC type I strains shown in Fig. 1. No type II RFLP patterns were obtained from these 24 isolates.

API Rapid Strep profiles. Four ATCC reference strains and 24 *S. uberis* isolates were examined for API Rapid Strep profiles. All 28 strains were either all positive (+) or negative (−) for acetyl methyl carbinol (+), hippurate hydrolysis (+), esculin hydrolysis (+), pyrrolidonylarylamidase (+), alkaline phosphatase (+), leucine arylamidase (+), arginine dehydrolase (+), ribose (+), L-arabinose (+), mannitol (+), sorbitol (+), lactose (+), trehalose (+), inulin (+), α-galactosidase (−), β-galactosidase (−), raffinose (−), starch (−), glycogen (−), and beta-hemolysis (−). All *S. uberis* ATCC type I strains and 21 *S. uberis* isolates were positive for β-glucuronidase, while *S. uberis* ATCC type II and 3 *S. uberis* isolates were β-glucuronidase negative. The three isolates which were β-glucuronidase negative had RFLP patterns identical to that of *S. uberis* type I strains (Fig. 2).

DISCUSSION

S. uberis is now recognized as an important causative agent of clinical and subclinical mastitis in dairy cows. The epidemiology of *S. uberis* is poorly understood. As early as 1979, Garvie and Bramley (6) suggested the existence of two distinct *S. uberis* genotypes. Since then, all attempts to differentiate the two genotypes by biochemical, serological, and genomic DNA fingerprinting have met with limited success.

Amplification of bacterial 16S rDNA by PCR has been accomplished with different species of bacteria (1, 24). The determination of the 16S rRNA sequence of *S. uberis* type I and II by Willams and Collins (23) has opened up avenues for more rapid and accurate determination of genotypic differences between the two types. On the basis of the 16S rRNA sequence reported by Willams and Collins (23), we used a modified broad-range eubacterial primer, 3′ p13B (3), while the 5′ primer was specific to *S. uberis*. Restriction sites were incorporated at the end of each primer to allow future cloning of the desired fragment. Amplification of the 16S rDNA fragment was achieved by PCR.

The amplified 16S rDNA fragment was digested with restriction endonucleases. Four restriction endonucleases were examined, and each could differentiate *S. uberis* type I from II. Results of the present study suggest that RFLP differences between the two genotypes of *S. uberis* exist. Thus, differentiation of *S. uberis* from *S. parauberis* can be achieved by PCR and RFLP analysis, as demonstrated in the present study.

TABLE 1. Predicted and observed 16S rDNA fragments of *S. uberis* types I and II by restriction endonuclease digestion

Restriction endonuclease	Restriction fragment length(s) (bp)			
	Type I		Type II	
	Predicted[a]	Observed[b]	Predicted	Observed
None	1,388[c]	1,400	1,388[c]	1,400
*Sca*I	1,388	1,400	164, 1,224	170, 1,230
*Nsp*HI	35, 140, 453, 760	140, 450, 760	35, 453, 900	450, 940
*Dde*I	24, 27, 67, 89, 94, 208, 434, 445	110, 200, 450[d]	24, 27, 67, 94, 297, 434, 445	110, 290, 450[d]
*Ava*II	222, 311, 855	230, 310, 860	61, 214, 1,113	230, 1,170

[a] Restriction fragment lengths predicted by IBI/Pustell sequence analysis (16).
[b] Restriction fragment lengths observed in 2% agarose gels.
[c] Uncut 16S rDNA size, representing the actual size of the fragment to be amplified, based on 16S rDNA sequence of Williams and Collins (23).
[d] Two fragments of similar size.

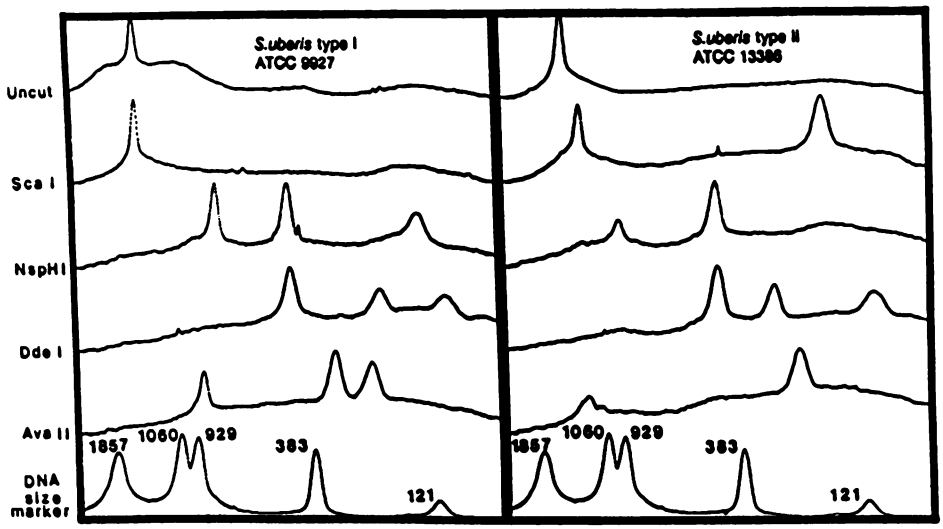

FIG. 3. Laser densitometric scans of 16S rDNA of *S. uberis type I* (ATCC 9927) and type II (ATCC 13386) strains, showing uncut 16S rDNA, a *Sca*I digest, an *Nsp*HI digest, a *Dde*I digest, and an *Ava*II digest. *Bst*NI-digested pBR322 DNA represents the DNA size marker (sizes in base pairs).

Analysis of API Rapid Strep profiles of *S. uberis* ATCC strain types I and II showed that strains differed from each other only with regard to the test for β-glucuronidase. Examination of API Rapid Strep profiles of 24 *S. uberis* isolates obtained from cows with clinical and subclinical mastitis showed that 3 isolates were similar to the *S. uberis* ATCC type II strain, while the remainder were similar to ATCC type I strains. Recently, Watts et al. (22) suggested that the test for β-glucuronidase could differentiate these two genotypes of *S. uberis*.

RFLP analysis of 16S rDNA of 24 *S. uberis* isolates showed that all isolates, including the 3 β-glucuronidase-negative strains, had RFLP patterns identical to that observed with *S. uberis* ATCC type I strains. This suggests that β-glucuronidase cannot be utilized for differentiating the two genotypes of *S. uberis* and contradicts observations of Watts et al. (22). In the absence of a suitable biochemical test, differentiation of *S. uberis* type I and II strains can best be achieved by RFLP analysis of amplified 16S rDNA, thus providing an alternative to laborious techniques such as DNA-DNA hybridization and 16S rRNA sequencing.

The 24 isolates of *S. uberis* obtained from bovine mammary secretions were selected randomly from our collection of 240 *S. uberis* strains isolated over a period of 5 years. The occurrence of strains similar to *S. uberis* type I in the two herds in Tennessee over a period of 5 years suggests the predominance of *S. uberis* type I strains. However, Garvie and Bramley (6) performed DNA-DNA hybridization with the two genotypes of *S. uberis* and demonstrated a frequent occurrence of *S. uberis* type II strains isolated from bovine mammary secretions in Great Britain. The occurrence of the two *S. uberis* genotypes could be due to different geographical areas and the predominance of a particular genotype in a herd. Use of the technique described in the present study will enable researchers to determine the prevalence of *S. parauberis* in dairy herds and delineate the importance of *S. uberis* and *S. parauberis* as etiologic agents of bovine mastitis.

In summary, differentiation of *S. uberis* type I from type II by RFLP analysis of PCR-amplified 16S rDNA was a reliable and reproducible method. This technique permitted differentiation of the two genotypes of *S. uberis* within 24 to 36 h. Using four different restriction endonucleases, we were able to demonstrate alterations in the DNA sequence of the 16S rRNA gene fragment of *S. uberis* types I and II. On the basis of our data demonstrating conserved multiple changes in rDNA sequence, we accept and support Williams and Collins's (23) proposal to designate *S. uberis* type II as *S. parauberis*.

ACKNOWLEDGMENTS

This investigation was supported by a University of Tennessee Professional Development Research Award, the Tennessee Agricultural Experiment Station, and the University of Tennessee College of Veterinary Medicine Center of Excellence Research Program in Livestock Diseases and Human Health.

We express our appreciation to Barbara Gillespie for technical assistance and to Sandra Dailey for clerical assistance.

REFERENCES

1. **Böddinghaus, B., T. Rogall, T. Flohr, H. Blöcker, and E. C. Böttger.** 1990. Detection and identification of mycobacteria by amplification of rRNA. J. Clin. Microbiol. **28**:1751–1759.
2. **Bramley, A. J., and F. H. Dodd.** 1984. Reviews of the progress of dairy science: mastitis control—progress and prospects. J. Dairy Res. **51**:481–512.
3. **Chen, K., H. Neimark, P. Rumore, and C. R. Steinman.** 1989. Broad range DNA probes for detecting and amplifying eubacterial nucleic acids. FEMS Microbiol. Lett. **57**:19–24.
4. **Diernhofer, K.** 1932. Aesculinbouillion als Hilfsmittel für die Differenzierung von Euter und Milchstreptokokken bei Massenuntersuchungen. Milchwirtschaftl. Forsch. **13**:368–378.
5. **Eberhart, R. J., and J. M. Buckalew.** 1977. Intramammary infections in a dairy herd with a low incidence of *Streptococcus agalactiae* and *Staphylococcus aureus* infections. J. Am. Vet. Med. Assoc. **171**:630–634.
6. **Garvie, I. E., and A. J. Bramley.** 1979. Streptococcus uberis: an approach to its classification. J. Appl. Bacteriol. **46**:295–304.
7. **Hill, A. W., and J. A. Leigh.** 1989. DNA fingerprinting of *Streptococcus uberis*: a useful tool for epidemiology of bovine mastitis. Epidemiol. Infect. **130**:165–171.
8. **Jayarao, B. M., S. P. Oliver, K. R. Matthews, and S. H. King.** 1991. Comparative evaluation of Vitek Gram-Positive identification system and API Rapid Strep system for identification of

Streptococcus species of bovine origin. Vet. Microbiol. **26**:301–308.

9. **Jayarao, B. M., S. P. Oliver, J. R. Tagg, and K. R. Matthews.** Genotypic and phenotypic analysis of *Streptococcus uberis* isolated from bovine mammary secretions. Epidemiol. Infect., in press.
10. **King, J. S.** 1981. *Streptococcus uberis*: a review of its role as a causative organism of bovine mastitis. I. Characteristics of the organisms. Br. Vet. J. **137**:36–51.
11. **King, J. S.** 1981. *Streptococcus uberis*: a review of its role as a causative organism of bovine mastitis. II. Control of infection. Br. Vet. J. **137**:160–165.
12. **Medlin, L. H., H. J. Elwood, S. Stickel, and M. L. Sogin.** 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA coding regions. Gene **71**:491–499.
13. **Oliver, S. P.** 1988. Frequency of isolation of environmental mastitis causing pathogens and incidence of new intramammary infections during the nonlactating period. Am. J. Vet. Res. **49**:1789–1793.
14. **Oliver, S. P., and B. A. Mitchell.** 1984. Prevalence of mastitis pathogens in herds participating in a mastitis control program. J. Dairy Sci. **67**:2436–2440.
15. **Oliver, S. P., and L. M. Sordillo.** 1988. Udder health in the periparturient period. J. Dairy Sci. **71**:2584–2606.
16. **Pustell, J., and F. C. Kafatos.** 1982. A convenient and adaptable package of DNA sequence analysis programs for microcomputers. Nucleic Acids Res. **10**:4765–4782.
17. **Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins.** 1990. The agent of bacillary angiomatosis: an approach to the identification of uncultured pathogens. N. Engl. J. Med. **323**:1573–1580.
18. **Roguinsky, M.** 1972. Réaction de *Streptococcus uberis* et de streptocoques voisins. Ouest Med. **25**:983–985.
19. **Smith, K. L., D. A. Todhunter, and P. S. Schoenberger.** 1985. Environmental pathogens and intramammary infection during the dry period. J. Dairy Sci. **68**:402–407.
20. **Sogin, M. L., J. H. Gunderson, H. J. Elwood, R. A. Alonso, and D. A. Peattie.** 1989. Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from *Giardia lamblia*. Science **243**:75–77.
21. **Watts, J. L.** 1987. Etiological agents and bovine mastitis. Vet. Microbiol. **6**:41–66.
22. **Watts, J. L., C. A. Chase, and R. J. Yancey, Jr.** 1991. Differentiation of *Streptococcus uberis* and *Streptococcus parauberis* isolated from bovine mammary glands, abstr. 18, p. 140. *In* Proceedings of the National Mastitis Council, National Mastitis Council, Inc., Arlington, Va.
23. **Williams, A. M., and M. D. Collins.** 1990. Molecular taxonomic studies on *Streptococcus uberis* types I and II. Description of *Streptococcus parauberis* sp. nov. J. Appl. Bacteriol. **68**:485–490.
24. **Wilson, K. H., R. B. Blitchington, and R. C. Greene.** 1990. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. J. Clin. Microbiol. **28**:1942–1946.