JOURNAL OF CLINICAL MICROBIOLOGY, May 1994, p. 1397–1401
0095-1137/94/$04.00+0

Vol. 32, No. 5

Identification and Characterization of *Serpulina hyodysenteriae* by Restriction Enzyme Analysis and Southern Blot Analysis

CHRIS SOTIROPOULOS,* PETER JOHN COLOE, AND STUART CRAIG SMITH

Biotechnology Unit, Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology University, Melbourne 3001, Australia

Received 19 July 1993/Returned for modification 7 September 1993/Accepted 4 February 1994

Chromosomal DNA restriction enzyme analysis and Southern blot hybridization were used to characterize *Serpulina hyodysenteriae* strains. When chromosomal DNAs from selected strains (reference serotypes) of *S. hyodysenteriae* were digested with the restriction endonuclease *Sau*3A and hybridized with a 1.1-kb *S. hyodysenteriae*-specific DNA probe, a common 3-kb band was always detected in *S. hyodysenteriae* strains but was absent from *Serpulina innocens* strains. When the chromosomal DNA was digested with the restriction endonuclease *Asp* 700 and hybridized with two *S. hyodysenteriae*-specific DNA probes (0.75 and 1.1 kb of DNA), distinct hybridization patterns for each *S. hyodysenteriae* reference strain and the Australian isolate *S. hyodysenteriae* 5380 were detected. Neither the 1.1-kb nor the 0.75-kb DNA probe hybridized with *Asp* 700- or *Sau*3A-digested *S. innocens* chromosomal DNA. The presence of the 3-kb *Sau*3A DNA fragment in *S. hyodysenteriae* reference strains from diverse geographical locations shows that this fragment is conserved among *S. hyodysenteriae* strains and can be used as a species-specific marker. Restriction endonuclease analysis and Southern blot hybridization with these well-defined DNA probes are reliable and accurate methods for species-specific and strain-specific identification of *S. hyodysenteriae*.

Serpulina hyodysenteriae is the etiological agent of swine dysentery (12, 35), causing mucohemorrhagic diarrhea in infected swine. The disease is prevalent worldwide (10), with a morbidity of up to 90% and a mortality of up to 30% in affected swine herds (11). The organism can be differentiated from the nonpathogenic swine intestinal spirochete *Serpulina innocens* by its ability to produce strong beta-hemolytic zones, enteropathogenicity test results, and serotyping results (2, 13, 14, 19).

Identification and characterization of *S. hyodysenteriae* serotypes are currently based on lipopolysaccharide (LPS) serotyping. Nine serotypes and various serotyping schemes have been proposed (2, 8, 9, 18, 19, 31). Serotyping is based on the extraction of the LPS from each *S. hyodysenteriae* isolate and determination of the reactivity of the LPS with hyperimmune rabbit antiserum raised to the whole cell of each reference isolate. Cross-reactivity between serotypes necessitates the additional step of cross-adsorption of rabbit antisera against other reference serotypes to obtain serotype-specific antisera (18, 19). Because of the complex antigenic relationship between the LPS antigens of the various serotypes, an alternative typing scheme for *S. hyodysenteriae* that is specific, easy to perform, and rapid is required (8, 9).

Recently, a new approach has been developed by using an LPS-directed monoclonal antibody produced to an Australian isolate, *S. hyodysenteriae* 8930, to characterize isolates, the result of which is the proposal of a new serotype (1). However, this method relies upon strain isolation prior to testing, as do conventional serotyping methods. Recent advances in recombinant DNA technology have led to the development of genotypic typing methods. Such methods, which include chromosomal DNA restriction endonuclease analysis (REA) (20, 39), plasmid profile analysis (29), and DNA probes (7, 36), may have application for typing *S. hyodysenteriae*.

Chromosomal DNA REA has proven to be a stable and reproducible method for strain differentiation and has been applied to many microorganisms, including *Staphylococcus epidermidis* (27), *Branhamella catarrhalis* (25), *Staphylococcus aureus* (3), and *Legionella pneumophila* (38). REA has been reported to be more effective than several other typing techniques such as phage typing (22, 39), serotyping (15, 21, 37), and antimicrobial susceptibility testing (25, 39) in differentiating between strains within serotypes (28). An REA typing system has been proposed for *S. hyodysenteriae* (5), but the technique appears to be cumbersome because the patterns have been difficult to compare because of the large number of fragments resolved by electrophoresis (5). Because of the complexities of these patterns, a more discriminatory method would be of value in characterizing *S. hyodysenteriae* strains. The use of Southern blot hybridization with a defined DNA probe would provide a clear means of distinguishing between strains. The DNA probes may be derived from a variety of sources including virulence determinants (16), randomly cloned fragments (30), whole chromosomal DNA (17), plasmids (26), and repetitive sequences (23, 24) and may represent valuable epidemiological markers. Because of the effectiveness of Southern blot analysis, it is seen as an alternative to current typing methods, including the less discriminatory serotyping method (20).

REA and Southern blot analysis were performed on chromosomal DNAs from different serotypes of *S. hyodysenteriae* 5380 and the *S. innocens* reference strain B256 (Table 1). *S. hyodysenteriae* 5380 was isolated from a naturally occurring swine dysentery outbreak in Victoria, Australia, and is serotypically different from the reference strains (1). Cultivation of the *Serpulina* spp. was performed as described previously (32). The two DNA probes (0.75 and 1.1 kb) specific for *S. hyodysenteriae* were previously developed in our laboratory (32) from virulent *S. hyodysenteriae* 5380 and, in the recombinant plasmid

* Corresponding author. Mailing address: Biochemistry and Molecular Biology Unit, School of Dental Science, University of Melbourne, Parkville 3000, Australia. Phone: 61-3-341-0296. Fax: 61-3-341-0339.

TABLE 1. Strains used in the Southern blot analysis

Strain[a]	Origin	Serotype	Reference
S. hyodysenteriae			
B78	United States	1	2
B204	United States	2	2
B169	Canada	3	2
A1	United Kingdom	4	2
B8044	United States	5	18
B6933	United States	6	18
5380	Australia	New	1
S. innocens B256	United States		13

[a] All strains are held at the Biotechnology Unit, Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology, Melbourne 3001, Australia.

forms (pBT9002 and pBT9003), were used to type the *S. hyodysenteriae* reference strains.

Genomic DNA was isolated by the procedure of Collins and Ross (4). The chromosomal DNA (1 to 2 μg) was digested with the restriction endonucleases *Asp* 700 (4.5 U/μg of DNA) and *Sau*3A (9 U/μg of DNA) for 3 h at 37°C in the buffer recommended by the manufacturer (Boehringer Mannheim, Sydney, Australia) and was heated at 65°C for 20 min to stop the reaction and to dissociate the reannealed fragments prior to electrophoresis. The digested DNA (20-μl sample volume) was electrophoresed at 35 V for 18 h in a horizontal 0.8% (wt/vol) agarose gel (BDH Chemicals) in a buffer containing 40 mM Tris-acetate and 10 mM EDTA (pH 8.3). After electrophoresis, the gels were stained with 0.5 μg of ethidium bromide per ml, illuminated with UV light, and photographed. For Southern blot analysis, the fractionated DNA in the gel was depurinated in 0.25 N HCl for 20 min, denatured in 0.5 M NaOH–1.5 M NaCl for 30 min, and neutralized in 0.5 M Tris-HCl–1.5 M NaCl–1 mM EDTA (pH 7.2) for 30 min. The DNA was transferred from the agarose gel to a 0.45-μm-pore-size nylon membrane (Hybond N; Amersham, Sydney, Australia) by the method of Southern (33). The filters were washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), air dried, UV cross-linked at 312 nm for 2.5 min, and used directly for hybridization as described previously (32). All hybridizations were performed under high-stringency conditions (65°C). The plasmids pBT9002 and pBT9003 containing the probes were radiolabelled by the oligo method (6) by using [α-^{32}P]dATP (Bresatec, Adelaide, Australia) to a specific activity of 10^6 to 10^7 cpm/mg as recommended by the manufacturer. Autoradiographs were exposed at −70°C for 1 to 3 days.

A variety of restriction endonucleases were tested for their capacities to restrict *S. hyodysenteriae* chromosomal DNA and provide clear and reproducible profiles. The restriction enzymes tested included *Hin*dIII, *Bgl*II, *Sma*I, *Bam*HI, *Eco*RI, *Hae*III, *Sau*3A, *Mbo*I, *Rsa*I, *Hha*I, *Pvu*II, and *Asp* 700 (data not shown). After careful examination, the restriction enzymes *Sau*3A and *Asp* 700 were used in the study because they produced the most definitive banding patterns and the clearest distinction of REA patterns between the *S. hyodysenteriae* reference strains. The REA profiles of *S. hyodysenteriae* and *S. innocens* genomic DNAs digested with either *Sau*3A or *Asp* 700 produced a large number of DNA fragments ranging in size from 40 to less than 0.5 kb (data not shown). The 1.1-kb DNA probe hybridized strongly to a 3.0-kb *Sau*3A fragment on all of the *S. hyodysenteriae* strains (Fig. 1A, lanes 1 and 3 to 8). No hybridization was observed with genomic DNA from *S. innocens* B256 (Fig. 1A, lane 2), which was consistent with earlier results obtained by using these probes in a colony blot hybridization (32).

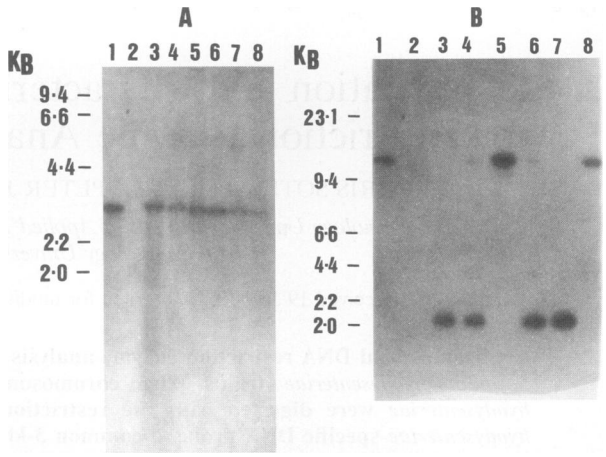

FIG. 1. Hybridization of the 1.1-kb probe to *Sau*3A (A) and *Asp* 700 (B) restriction-digested genomic DNAs from *Serpulina* spp. Lanes: 1, *S. hyodysenteriae* 5380; 2, *S. innocens* B256; 3, *S. hyodysenteriae* B78; 4, *S. hyodysenteriae* B204; 5, *S. hyodysenteriae* B169; 6, *S. hyodysenteriae* A1; 7, *S. hyodysenteriae* B8044; 8, *S. hyodysenteriae* B6933. Fragment sizes (in kilobases) are indicated on the left.

When the genomic DNAs of *S. hyodysenteriae* reference strains, *S. hyodysenteriae* 5380, and *S. innocens* B256 were digested with *Asp* 700, *S. hyodysenteriae* strains could be divided into two groups after hybridization with the 1.1-kb DNA probe. *S. hyodysenteriae* serotype reference strains B78, B204, A1, and B8044 hybridized to a 2.0-kb band (Fig. 1B, lanes 3, 4, 6, and 7). *S. hyodysenteriae* reference strains B169 and B6933 and the Australian isolate *S. hyodysenteriae* 5380 hybridized to a large 15-kb fragment (Fig. 1B, lanes 1, 5, and 8). No hybridization was observed with *S. innocens* B256 (Fig. 1B, lane 2).

Hybridization of *Asp* 700-digested genomic DNA from the *S.*

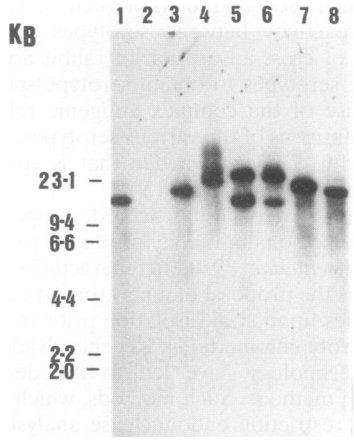

FIG. 2. Hybridization of the 0.75-kb DNA probe to *Asp* 700 restriction-digested genomic DNAs from *Serpulina* spp. Lanes: 1, *S. hyodysenteriae* 5380; 2, *S. innocens* B256; 3, *S. hyodysenteriae* B78; 4, *S. hyodysenteriae* B204; 5, *S. hyodysenteriae* B169; 6, *S. hyodysenteriae* A1; 7, *S. hyodysenteriae* B8044; 8, *S. hyodysenteriae* B6933. Fragment sizes (in kilobases) are indicated on the left.

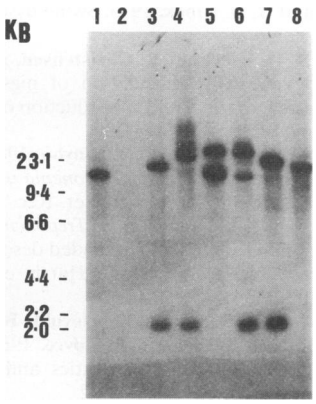

FIG. 3. Hybridization of both DNA probes (0.75 and 1.1 kb) to *Asp* 700 restriction-digested genomic DNAs from *Serpulina* spp. Lanes: 1, *S. hyodysenteriae* 5380; 2, *S. innocens* B256; 3, *S. hyodysenteriae* B78; 4, *S. hyodysenteriae* B204; 5, *S. hyodysenteriae* B169; 6, *S. hyodysenteriae* A1; 7, *S. hyodysenteriae* B8044; 8, *S. hyodysenteriae* B6933. Fragment sizes (in kilobases) are indicated on the left.

hyodysenteriae strains and *S. innocens* B256 with the 0.75-kb DNA probe produced different hybridization patterns (Fig. 2, lanes 1 and 3 to 8). The 0.75-kb DNA probe hybridized only to one band on the chromosomal DNA of *S. hyodysenteriae* 5380, producing a 15-kb DNA band (Fig. 2, lane 1). *S. hyodysenteriae* B78 produced a 21-kb DNA band (Fig. 2, lane 3). *S. hyodysenteriae* B204 produced a strong 23-kb DNA band and extra higher-molecular-size bands which could be accounted for as incomplete digestion of the chromosomal DNA (Fig. 2, lane 4). *S. hyodysenteriae* B8044 produced a 22-kb DNA band (Fig. 2, lane 6), and *S. hyodysenteriae* B6933 produced a 19-kb DNA band (Fig. 2, lanes 1 and 8). The 0.75-kb DNA probe hybridized to two identical DNA bands of 25 and 16 kb (Fig. 2, lanes 5 and 6) in *S. hyodysenteriae* B169 and A1. No hybridization was observed with *S. innocens* B256 genomic DNA (Fig. 2, lane 2).

S. hyodysenteriae and *S. innocens* chromosomal DNAs were digested with *Asp* 700 and hybridized in tandem with radiolabelled 0.75- and 1.1-kb DNA probes. Each *S. hyodysenteriae* strain could be differentiated clearly. *S. hyodysenteriae* 5380 produced a 15-kb band (Fig. 3, lane 1), *S. hyodysenteriae* B78 produced 21- and 2.0-kb bands (Fig. 3, lane 3), and *S. hyodysenteriae* B204 produced 23- and 2.0-kb bands (Fig. 3, lane 4). *S. hyodysenteriae* B169 produced 25- and 16-kb DNA bands (Fig. 3, lane 5); *S. hyodysenteriae* A1 produced three bands of 25, 16, and 2.0 kb (Fig. 3, lane 6). *S. hyodysenteriae* B8044 produced 22- and 2.0-kb bands (Fig. 3, lane 7). *S. hyodysenteriae* B6933 produced a 19-kb DNA band (Fig. 3, lane 8). The DNA probes did not hybridize to *Asp* 700-digested *S. innocens* B256 DNA (Fig. 3, lane 2).

DNA hybridization studies have been used for the detection, classification, characterization, taxonomic evaluation, and evolutionary studies of bacteria (36). A variety of restriction endonucleases were analyzed for their capacities to digest *S. hyodysenteriae* genomic DNA. The use of the restriction enzyme *Asp* 700 (recognition sequence, GAANN/NNTTC) provided the least number of bands, and hence, the resolution and distinct reproducible DNA banding profiles were enhanced. Total digests of *S. hyodysenteriae* chromosomal DNA with *Asp* 700 enabled subtle variations in DNA restriction enzyme patterns to be detected and enhanced the differentiation between the *S. hyodysenteriae* strains examined and *S. innocens* B256 (data not shown). *S. hyodysenteriae* reference strains could be differentiated by the polymorphisms in the restriction enzyme patterns present in the higher-molecular-size region (Fig. 1B, 2, and 3). These chromosomal REA patterns remained stable after subculturing of *S. hyodysenteriae* strains repeatedly over a 2-year period (data not shown).

Whole *S. hyodysenteriae* 5380 chromosomal DNA could not be used as a probe to identify and differentiate *S. hyodysenteriae* strains because of the high DNA-DNA homology within *S. hyodysenteriae* strains and the 28% DNA-DNA homology with the swine intestinal commensal organism *S. innocens*, which is commonly found in up to 30% of swine (14, 34). The *S. hyodysenteriae* group is not as heterologous as has been observed with other spirochetes such as *Borrelia burgdorferi* (17). Thus, it appears that *S. hyodysenteriae* strains examined from diverse geographical sources are a closely related group at the DNA homology level. The DNA profiles produced by REA in conjunction with Southern blot analysis with DNA probes (0.75 and 1.1 kb of DNA) were less complex than visual analysis of the *Asp* 700-digested chromosomal DNA (data not shown) or the *Hae*III-*Hin*dIII patterns reported by Combs et al. (5) in a study of a proposed *S. hyodysenteriae* typing system based on REA.

The 1.1-kb DNA probe hybridized with a 3.0-kb DNA fragment of *Sau*3A-restricted *S. hyodysenteriae* chromosomal DNA but not with *S. innocens* chromosomal DNA. The 3.0-kb *Sau*3A DNA fragment was observed in all *S. hyodysenteriae* reference strains and the virulent Australian isolate *S. hyodysenteriae* 5380. The reference strains are derived from diverse geographical sources, and thus, the observation of a single DNA band indicates a conserved region in the genomes of members of this species. It is proposed that this marker may be useful for the species-specific but not serotype-specific identification of *S. hyodysenteriae*, although further evaluation against other species found in the swine intestine, e.g., *Treponema succinofaciens*, needs to be done before a general claim of specificity can be validated.

The application of either the 1.1-kb DNA probe or the 0.75-kb DNA probe to *Asp* 700-digested *Serpulina* sp. genomic DNA demonstrates that the probes are species specific because they do not recognize *S. innocens* and can be used to subdivide the *S. hyodysenteriae* reference serotype strains into different groups (Fig. 1B and 2). The reference serotype strains B169 and B6933 and *S. hyodysenteriae* 5380 could be distinguished from strains of the other serotypes (B78, B204, A1, B8044) by using the 1.1-kb DNA probe. The application of the 0.75-kb DNA probe to *Asp* 700-digested *S. hyodysenteriae* chromosomal DNA enabled the distinction of each *S. hyodysenteriae* reference strain except for the serotype 3 (B169) and serotype 4 (A1) reference strains and strains of serotype 1 (B78) and serotype 6 (B6933), which produced identical DNA banding profiles. Therefore, although the Southern blot analysis banding profiles produced by using either the 0.75- or the 1.1-kb DNA probe could group some *S. hyodysenteriae* reference strains and differentiate between other serotype reference strains, each reference strain did not produce a distinctive banding profile. However, the simultaneous use of both the 1.1- and 0.75-kb DNA probes in the Southern blot analysis could clearly differentiate all of the *S. hyodysenteriae* serotype reference strains and the Australian isolate 5380 (Fig. 3).

Originally, we classified *S. hyodysenteriae* 5380 as serotype 4 on the basis of the LPS serotyping scheme of Baum and Joens (2) and Mapother and Joens (19). However, Alderton et al. (1) have shown recently that this strain is recognized by an LPS-specific monoclonal antibody which does not recognize the reference strains of serotypes 1 to 6, including the refer-

ence strain of serotype 4 (A1) used in the present study. The Southern blot analysis described in this report also clearly demonstrates that *S. hyodysenteriae* 5380 can be distinguished from the reference strains of the known *S. hyodysenteriae* serotypes, since it produces a different hybridization pattern (Fig. 3).

The Southern hybridization protocols with the two *S. hyodysenteriae*-specific DNA probes reported here could be used to provide crucial epidemiological and typing information on *S. hyodysenteriae* in swine, including whether new strains may evolve by DNA rearrangement or transposon or bacteriophage integration at specific sequences which may lead to restriction fragment length polymorphisms in specific DNA regions. The Southern hybridization pattern could also be used to detect and trace the transmission of pathogenic strains of *S. hyodysenteriae* in outbreaks of disease, distinguish isolates from various sources that may be responsible for outbreaks of swine dysentery, and provide a method of characterizing organisms excreted in any vaccination and subsequent challenge trials.

The use of two DNA probes together for characterizing *S. hyodysenteriae* strains would be neither time-consuming nor expensive, and the application of nonisotopic labelling techniques enables Southern blot analysis to be performed safely and readily in diagnostic and research laboratories.

A large number of *S. hyodysenteriae* strains need to be characterized by REA and REA-Southern blot analysis with the two DNA probes (0.75 and 1.1 kb) in order to determine the efficacy of this molecular typing system for application to epidemiological investigations. The present study has provided evidence that REA of *S. hyodysenteriae* strains with *Asp* 700 identifies genomic variations among the *S. hyodysenteriae* strains and that these variations can be used to differentiate strains of the reference serotypes. REA in association with the use of DNA probes provides a specific and sensitive method for the identification and characterization of *S. hyodysenteriae* strains and could be used to provide an alternative to current identification and characterization methods.

REFERENCES

1. **Alderton, M. R., S. C. Smith, and P. J. Coloe.** 1993. Identification of a novel group of *Serpulina hyodysenteriae* isolates by using a lipopolysaccharide-specific monoclonal antibody. J. Clin. Microbiol. **31**:1326–1328.
2. **Baum, D. H., and L. A. Joens.** 1979. Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect. Immun. **25**:792–796.
3. **Carles-Nurit, M. J., B. Christophle, S. Broche, A. Gouby, N. Bouzies, and M. Ramuz.** 1992. DNA polymorphisms in methicillin-susceptible and methicillin-resistant strains of *Staphylococcus aureus*. J. Clin. Microbiol. **30**:2092–2096.
4. **Collins, D. M., and D. E. Ross.** 1984. Restriction endonuclease analysis of *Campylobacter* strains with particular reference to *Campylobacter fetus* ss *fetus*. J. Med. Microbiol. **18**:117–124.
5. **Combs, B., D. J. Hampson, J. R. L. Mhoma, and J. R. Buddle.** 1989. Typing of *Treponema hyodysenteriae* by restriction endonuclease analysis. Vet. Microbiol. **19**:351–359.
6. **Feinberg, A. P., and B. Volgstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
7. **Garaizar, J., M. E. Kaufmann, and T. L. Pitt.** 1991. Comparison of ribotyping with conventional methods for the type identification of *Enterobacter cloacae*. J. Clin. Microbiol. **29**:1303–1307.
8. **Hampson, D. J., J. R. L. Mhoma, and B. Combs.** 1989. Analysis of lipopolysaccharide antigens of *Treponema hyodysenteriae*. Epidemiol. Infect. **103**:275–284.
9. **Hampson, D. J., J. R. L. Mhoma, B. Combs, and J. R. Buddle.** 1989. Proposed revisions to the serological typing system for *Treponema hyodysenteriae*. Epidemiol. Infect. **102**:75–84.
10. **Harris, D. L.** 1974. Current status on research on swine dysentery. J. Am. Vet. Med. Assoc. **164**:809–812.
11. **Harris, D. L., and R. D. Glock.** 1973. Swine dysentery. Vet. Scope **17**:2–7.
12. **Harris, D. L., R. D. Glock, C. R. Christensen, and J. M. Kinyon.** 1972. Swine dysentery. I. Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. Vet. Med. Small Anim. Clin. **67**:61–64.
13. **Joens, L. A., R. D. Glock, and J. M. Kinyon.** 1980. Differentiation of *Treponema hyodysenteriae* from *Treponema innocens* by enteropathogenicity testing in CF1 mouse. Vet. Rec. **107**:527–529.
14. **Kinyon, J. M., and D. L. Harris.** 1979. *Treponema innocens*, a new species of intestinal bacteria, and amended description of the type strains of *Treponema hyodysenteriae* Harris et al. Int. J. Syst. Bacteriol. **29**:102–109.
15. **Kristiansen, B.-E., K. W. Lind, K. Mevold, B. Sørensen, L. O. Frøholm, K. Bryn, T. Tjade, and K. Bøvre.** 1988. Meningococcal phenotypic and genotypic characteristics and human antibody levels. J. Clin. Microbiol. **26**:1988–1992.
16. **Lanser, J. A., and P. A. Anargyros.** 1985. Detection of *Escherichia coli* adhesins with DNA probes. J. Clin. Microbiol. **22**:425–427.
17. **LeFebvre, R. B., G. C. Perng, and R. C. Johnson.** 1989. Characterization of *Borrelia burgdorferi* isolates by restriction endonuclease analysis and DNA hybridization. J. Clin. Microbiol. **27**:636–639.
18. **Li, Z., M. Belanger, and M. Jacques.** 1991. Serotyping of Canadian isolates of *Treponema hyodysenteriae* and description of the two new serotypes. J. Clin. Microbiol. **9**:2794–2797.
19. **Mapother, M. E., and L. A. Joens.** 1985. New serotypes of *Treponema hyodysenteriae*. J. Clin. Microbiol. **22**:161–164.
20. **Marshall, R. B., P. J. Winter, and R. Yanagawa.** 1984. Restriction endonuclease DNA analysis of *Leptospira interrogans* serovars *icterohaemorrhagiae* and *hebdomadis*. J. Clin. Microbiol. **20**:808–810.
21. **Mogollon, J. D., C. Pigdoan, M. P. Murtaugh, J. E. Collins, and P. P. Cleary.** 1991. Identification of epidemic strains of *Streptococcus suis* by genomic fingerprinting. J. Clin. Microbiol. **29**:782–787.
22. **Nocera, D., E. Bannermann, J. Roccourt, K. Jaton-Ogay, and J. Bille.** 1990. Characterization by DNA restriction enzyme analysis of *Listeria monocytogenes* strains related to the Swiss epidemic of listeriosis. J. Clin. Microbiol. **28**:2259–2263.
23. **Otal, I., C. Martin, V. Vincent-Leug-Frebault, D. Thierry, and B. Gicquel.** 1991. Restriction fragment length polymorphism analysis using IS*6110* as an epidemiological marker in tuberculosis. J. Clin. Microbiol. **29**:1252–1254.
24. **Pacciarini, M. L., M. L. Savio, S. Tagliabue, and C. Ross.** 1992. Repetitive sequences cloned from *Leptospira interrogans* serovar hardjo genotype hardjoprajitno and their application to serovar identification. J. Clin. Microbiol. **30**:1243–1249.
25. **Patterson, J. E., T. F. Patterson, P. Faurel, W. J. Hierholzer, Jr., and M. J. Zervos.** 1989. Evaluation of restriction endonuclease analysis as an epidemiologic typing system for *Branhamella catarrhalis*. J. Clin. Microbiol. **27**:944–946.
26. **Renaud, F., J. Etienne, A. Bertrand, Y. Bruno, T. B. Greenland, J. Freney, and J. Fleurette.** 1991. Molecular epidemiology of *Staphylococcus haemolyticus* strains isolated in an Albanian hospital. J. Clin. Microbiol. **29**:1493–1497.
27. **Renaud, F., J. Freney, J. Etienne, M. Bes, Y. Brun, O. Barsotti, S. Andre, and J. Fleurette.** 1988. Restriction endonuclease analysis of *Staphylococcus epidermidis* DNA may be used as a useful epidemiological marker. J. Clin. Microbiol. **26**:1729–1734.
28. **Robinson, A. J., P. Ramadass, A. Lee, and R. B. Marshall.** 1982. Differentiation of subtypes within *Leptospira interrogans* serovars *hardjo*, *balanica*, and *tarassovi*, by bacterial restriction endonuclease DNA analysis (BRENDA). J. Med. Microbiol. **15**:331–338.
29. **Schwan, T. G., W. J. Simpson, M. E. Schrumpf, and R. H. Karstens.** 1989. Identification of *Borrelia burgdorferi* and *B. hermsii* using DNA hybridization probes. J. Clin. Microbiol. **27**:1734–1738.
30. **Sirois, M., E. G. Lemire, and R. C. Levesque.** 1991. Construction of a DNA probe and detection of *Actinobacillus pleuropneumoniae* by using polymerase chain reaction. J. Clin. Microbiol. **29**:1183–1187.
31. **Smith, S. C., F. Roddick, S. Ling, N. L. Gerraty, and P. J. Coloe.** 1990. Biochemical and immunochemical characterisation of

strains of *Treponema hyodysenteriae*. Vet. Microbiol. **24:**29–41.
32. **Sotiropoulos, C., S. C. Smith, and P. J. Coloe.** 1993. Characterization of two DNA probes to *Serpulina hyodysenteriae*. J. Clin. Microbiol. **31:**1746–1752.
33. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–518.
34. **Stanton, T. B., N. S. Jensen, T. A. Casey, L. A. Tordoff, F. E. Dewhirst, and B. J. Paster.** 1991. Reclassification of *Treponema hyodysenteriae* and *Treponema innocens* in a new genus, *Serpula* gen. nov., as *Serpula hyodysenteriae* comb. nov. and *Serpula innocens* comb. nov. Int. J. Syst. Bacteriol. **41:**50–58.
35. **Taylor, D. J., and T. J. L. Alexander.** 1971. The production of dysentery in swine feeding cultures containing a spirochete. Br. Vet. J. **127:**58–61.
36. **Tenover, F. C.** 1988. Diagnostic deoxyribonucleic acid probes for detection of infectious diseases. Clin. Microbiol. Rev. **1:**82–101.
37. **Thiermann, A. B., and R. B. LeFebvre.** 1989. Restriction enzyme analysis and their molecular techniques in identification and classification of *Leptospira* and other pathogens of veterinary importance, p. 145–184. *In* B. Swaminathan and G. Prakash (ed.), Nucleic acid and monoclonal antibody probes: applications in diagnostic microbiology. Marcel Dekker, Inc., New York.
38. **Tompkins, L. S., N. J. Troup, T. Woods, W. Bibb, and R. M. McKinney.** 1987. Molecular epidemiology of *Legionella* species by restriction endonuclease and allozyme analysis. J. Clin. Microbiol. **10:**1875–1880.
39. **Tveten, Y., B. E. Kristiansen, E. Aske, A. Jenkins, and T. Hofstad.** 1991. DNA fingerprinting of isolates of *Staphylococcus aureus* from newborns and their contacts. J. Clin. Microbiol. **29:**1100–1105.