Characterization of *Serpulina hyodysenteriae* Isolates of Serotypes 8 and 9 from Quebec by Restriction Endonuclease Fingerprinting and Ribotyping

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ABSTRACT

This study was undertaken to assess the discriminatory value of restriction endonuclease fingerprinting (REF) analysis and ribotyping of 21 *Serpulina hyodysenteriae* isolates of serotypes 8 and 9. For REF analysis, DNAs were digested with the *Bgl*II restriction enzyme and the resultant fragments were separated by polyacrylamide gel electrophoresis. For ribotyping, hybridization of *Bgl*II genomic fragments with a probe of *rrn*B operon using an *Escherichia coli* rDNA probe was performed on all isolates. Although many isolates shared a common pattern by *Bgl*II REF and *Bgl*II ribotyping analysis, differences among some *S. hyodysenteriae* isolates were observed. REF and ribotyping using *Bgl*II restriction enzyme, were not specific for serotypes. The predominance of an REF and a ribotype pattern among *S. hyodysenteriae* isolates from Quebec suggested that epidemiologically important *S. hyodysenteriae* types occur in different swine herds.

RÉSUMÉ

Vingt et un isolats de *Serpulina hyodysenteriae* appartenant aux sérotypes 8 et 9 ont été analysés par empreinte génomique après digestion à l'aide d'une enzyme de restriction et ribotypage. Pour procéder à l'analyse de l'empreinte génomique, l'ADN des isolats a été digéré avec l'enzyme de restriction *Bgl*II, puis les fragments ont été séparés par électrophorèse sur gel de polyacrylamide. Pour procéder à l'analyse par ribotypage des isolats, les fragments d'ADN générés par digestion avec l'enzyme de restriction *Bgl*II ont été hybridés avec une sonde recouvrant l'opéron *rrn*B. Bien que plusieurs isolats ont révélé un patron d'empreinte génomique et de ribotypage similaire, des variations génomiques ont été observées parmi certains isolats. Par contre, ni l'empreinte génomique ni le ribotypage n'ont permis de différencier les isolats selon leur sérotype. Il a été remarqué qu'un patron d'empreinte génomique et de ribotype était prédominant. Ceci indique que des isolats importants épidémiologiquement seraient retrouvés dans plusieurs élevages.

Serpulina (*Treponema*) *hyodysenteriae*, a gram-negative anaerobic spirochete, is the causative agent of swine dysentery, a mucohemorrhagic diarrheal disease in which lesions are confined to the large intestine of pigs. Nine serotypes of *S. hyodysenteriae* have been described on the basis of agar gel double immunodiffusion precipitation with extracted LPS and hyperimmune rabbit antisera (1, 2, 3). Serogrouping of *S. hyodysenteriae* has also been proposed (4, 5).

Different methods for measuring genetic relatedness have been employed with *S. hyodysenteriae* including DNA-DNA association, rDNA sequence homology, and multilocus enzyme electrophoresis (6, 7, 8, 9). Restriction endonuclease fingerprinting (REF) had been found to be very useful in epidemiological studies of other clinically important bacteria (10, 11). Ribotyping has also been used to study relatedness of different bacterial species (12, 13, 14, 15). REF with *Taq*I restriction enzyme of Dutch pathogenic *S. hyodysenteriae* isolates resulted in two main patterns while nonpathogenic isolates all had distinct patterns, and two main patterns of *S. hyodysenteriae* were observed using hybridization with Fla and Tly probes corresponding to flagellar and hemolysin DNA sequences, respectively (16). Isolates of *S. hyodysenteriae* from herds in Australia fell into eight serogroups and showed distinct REF patterns (9). The *Sau*3A rDNA gene restriction patterns were strongly conserved among *S. hyodysenteriae* isolates (17, 18). However, several *S. hyodysenteriae* strains were different from each other when the restriction enzymes *Ssp*I or *Bgl*II were used (17).

We reported two new serotypes of *S. hyodysenteriae*, serotypes 8 and 9 (3). These serotypes represented 70% of the isolates and were the major serotypes found in the province of Quebec, Canada. The purpose of this study was to evaluate genetic relatedness and diversity in *S. hyodysenteriae* serotypes 8 and 9 isolates associated with swine dysentery in Quebec. Isolates were studied by REF analysis using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and by ribotyping using an *E. coli* rDNA probe, after *Bgl*II restriction enzyme digestion.

The reference strains representing serotype 8 (FM 88-90 or ATCC 49887) and serotype 9 (FMV 89-3323 or ATCC 49886) of *S. hyodysenteriae* as well as 19 isolates (13 of serotype 8, 6 of serotype 9) from 13 swine herds in the Saint-Hyacinthe area (Quebec) were isolated from cases of swine dysentery in 1988 and 1989 (3) (Table I). Bacteria were grown on blood agar base no.2 (Oxoid Ltd, Hampshire, England) containing 5% bovine blood. Plates were incubated anaerobically at 37°C for four days in jars using GasPak Plus genera-

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TABLE I. Classification of *S. hyodysenteriae* isolates of serotypes 8 and 9 by REF on SDS-PAGE and by ribotyping

Herd	Isolate	REF pattern	Ribotype
Serotype 8			
1	FM88-76	B	B
	FM88-116	A	A
	FM88-95 P6	A	A
	FM88-106P6	A	A
2	FM88-104	A	A
3	FM88-105P9	A	A
4	FM88-81P6	A	A
	FM88-77P6	A	A
5	FM88-90[a]	A	A
	FM88-91	A	A
6	FM88-86P7	D	D
	FM88-88P10	C	C
7	FM88-89	A	A
8	FM88-94	A	A
Serotype 9			
9	FMV89-3323[a]	E	A
10	FMV89-1614D	F	C
11	FMV89-1020P4	G	A
12	89-1066D81	B	B
	89-1066C21	E	A
	89-1066C2	E	A
	1389-440	G	A

[a]Indicates the references strains

tor atmosphere (BBL, Beckton Dickinson and Co., Cockeysville, Maryland).

Total cellular DNA was isolated using a modification of the method of Leblanc and Lee (19) as described (15). Bacterial cells were washed in PBS, lysed, and DNAs were extracted with phenol-chloroform. The DNAs were then dissolved in TE (10 mM Tris/HCl pH 8.0, 0.1 M EDTA pH 8.0) and treated with RNAse A (10 mg mL-1). Concentrations of the DNAs were determined spectrophotometrically (20).

Isolates were characterized by restriction endonuclease fingerprinting (REF) and rDNA gene restriction fragment length (ribotyping) using *Bgl*II restriction enzyme to digest genomic DNA. DNAs were digested separately using a fourfold excess of *Bgl*II according to the manufacturer's instructions (Pharmacia (Canada) Inc., Baie d'Urfé, Québec).

For REF, fragments were resolved on a 7.5% SDS-polyacrylamide gel and visualized by silver staining using a modification of the method of Sammons *et al* (21) as described (15). The gels were photographed or stored in 25% ethanol/10% acetic acid (v/v). Gels were then inspected visually and each different profile was assigned a letter. The REF profiles were determined in at least two independent gels. Approximately 20 fragments, ranging in size from 0.4 to 1.6 kb, were analyzed when DNAs were digested with *Bgl*II (Figs. 1A and 1B). Although REF patterns were similar, differences were detected with bands in the 0.7, 0.9 and 1.6 kb area (Fig. 1B). Restriction endonuclease fingerprinting pattern A was most common (12/21 isolates). Even though they were from different herds, most isolates of serotype 8 belonged to REF pattern group A, suggesting these strains were more epidemiologically and clinically important. The REF profiles of serotype 8 strains FM88-76 (Fig. 1A, lane 1), FM88-86P7 (Fig. 1A, lane 11) and FM88-88P10 (Fig. 1B, lane 1) were quite different from the others. The REF profiles of serotype 9 strains were heterogeneous and many strains had patterns similar to pattern A but with some variations (Fig. 1B). Some isolates of serotype 9 from different herds shared similar REF profile, but were different from serotype 8 profiles with the exception of serotype 8 strain FM88-76 (Fig. 1A, lane 1), and of serotype 9 strain 89-1066D81 (Fig. 1B, lane 7). Isolates of *S. hyodysenteriae* from herds in Australia had distinct REF patterns were recognized within serogroups whilst a few isolates with identical REF pattern were from different serogroups (9).

For ribotyping analysis of *S. hyodysenteriae* DNA *Bgl*II restriction digests of genomic DNA, were prepared using 3 μg of DNA in a total volume of 50 μL. Approximately 1 μg of digested DNA per lane was applied in 0.8 % (w/v) agarose gel in Tris/acetate buffer (40 mM Tris/acetate pH 8.3, 2 mM EDTA) at 30 v for 16 h. DNA fragments were transferred to nylon membranes and the membranes were hybridized with alpha^{32}P labelled *E. coli* rDNA as described previously (15). This rDNA probe from pKK3535 (22) comprising a 7.7 kb *Bam*HI fragment was isolated by gel purification (20) and end-labelled by random priming (Pharmacia) with (^{32}P)dCTP. The hybridization of *Bgl*II fragments with a probe of *rrnB* operon gave few fragments, thereby making comparisons easier.

Based on their pattern of *Bgl*II bands, many isolates showed similar ribotype patterns. Four ribotypes were observed among the 21 isolates of *S. hyodysenteriae* representing serotypes 8 and 9 but there was no correlation between the serotype of the isolate and the ribotype (Table I). Ribotype A represented the most common type in isolates of both serotypes (16/21 isolates) with bands present in the region of 9, 7.2, and 4.4 kb (Figs. 2A and 2B). The presence of a 1.7 kb band and the absence of a 9 kb were the differences between ribotype A and ribotype D.

The low number of bands makes ribotyping analysis easier; but may overlook some differences. The use of SDS-PAGE REF analysis and silver staining allowed better pattern resolutions than REF from agarose gels (data not shown).

It has been shown that *Sau*3A ribotypes of *S. hyodysenteriae* are strongly conserved (17). Thus, the choice of enzyme for ribotyping may be critical. We found that *Bgl*II discriminated the *S. hyodysenteriae* isolates. However, when comparing REFs and ribotyping it can be concluded that SDS-PAGE allows a finer differentiation between isolates, and the homogeneity found by ribotyping suggests that most isolates are genetically similar. The techniques are complementary and each of them can reveal differences not seen by the other, particularly for serotype 9 isolates. It is interesting to note that the serotype 8 isolates fell into the same pattern for both techniques (Table I).

Although serotyping is a widely used technique for epidemiological studies, it cannot definitively determine if

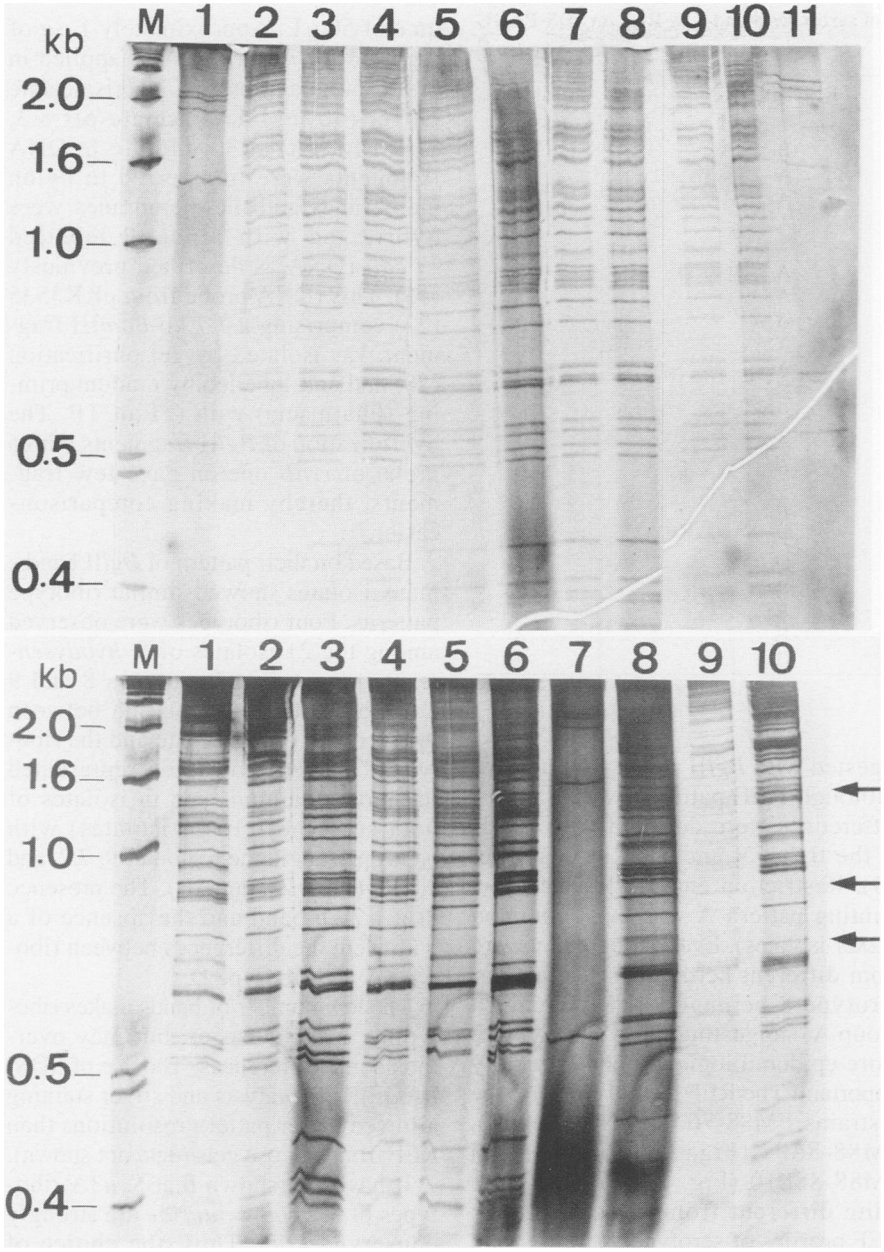

Fig. 1. REF profiles of *Bgl*II digested DNAs of *S. hyodysenteriae* serotype 8 isolates and serotype 9 isolates.
Panel A (serotype 8 isolates). Lanes: 1, FM88-76; 2, FM88-116; 3, FM88-95P6; 4, FM88-106P6; 5, FM88-104; 6, FM88-105P9; 7, FM88-81P6; 8, FM88-77P6; 9, FM88-90; 10, FM88-91; 11, FM88-86P7.
Panel B [serotype 8 isolates (lanes 1 to 3) and serotype 9 isolates (lanes 4 to 10)]. Lanes: 1, FM88-88P10; 2, FM88-89; 3, FM88-94, 4, FMV89-3323; 5, FMV89-1614D; 6, FMV89-1020P4; 7, 89-1066D81; 8, 89-1066C21; 9, 89-1066C2; 10, 89-440.
The size of the molecular markers are indicated in kb; the arrows indicate the positions where major differences in the REF profiles were noted.

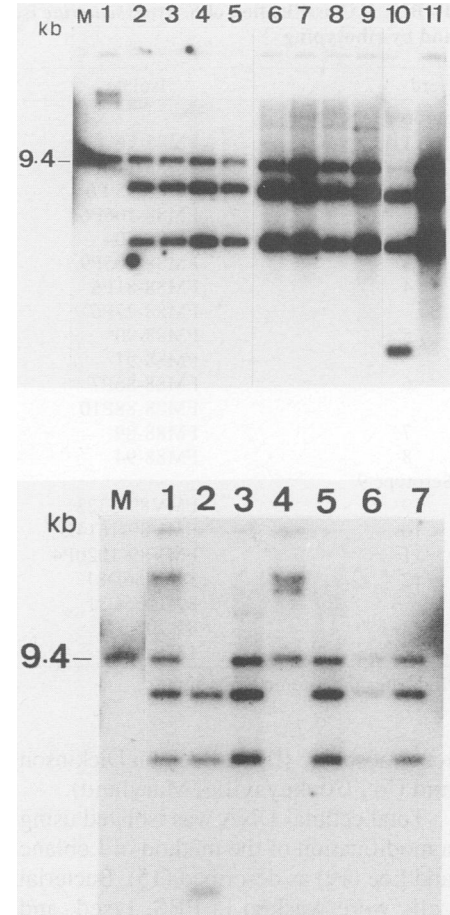

Fig. 2. Southern blot of *Bgl*II digested DNAs from *S. hyodysenteriae* serotype 8 isolates (panel A) and serotype 9 isolates (panel B). The blots were probed with alpha (^{32}P)-labelled rDNA probe.
Panel A. Lanes: 1, FM88-76; 2, FM88-116; 3, FM88-95P6; 4, FM88-106P6; 5, FM88-104; 6, FM88-81P6; 7, FM88-77P6; 8, FM88-90; 9, FM88-91; 10, FM88-88P10; 11, FM88-89.
Panel B. Lanes: 1, FMV89-3323; 2, FMV89-1614D; 3, FMV89-1020P4; 4, 89-1066D81; 5, 89-1066C21; 6, 89-1066C2; 7, 89-440. The size of the molecular marker is indicated in kb.

isolates are epidemiologically related or unrelated isolates. The comparison of the restriction patterns and ribotypes showed a genetic conservation with some diversity among *S. hyodysenteriae* isolates belonging to serotypes 8 and 9. Although these results must be confirmed with a larger number of herds and animals, the predominance of a particular REF and ribotype pattern among *S. hyodysenteriae* isolates in Quebec suggest that epidemiologically important *S. hyodysenteriae* isolates occur in different swine herds. This information could be useful in terms of vaccine development.

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REFERENCES

1. **BAUM DH, JOENS LA.** Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect Immun 1979; 25: 792–796.
2. **MAPOTHER ME, JOENS LA.** New serotypes of *Treponema hyodysenteriae*. J Clin Microbiol 1985; 22: 161–164.

3. **Li Z, BELANGER M, JACQUES M.** Serotyping of canadian isolates of *Treponema hyodysenteriae* and description of two new serotypes. J Clin Microbiol 1991; 29: 2794–2797.
4. **HAMPSON DJ, MHOMA JRL, COMBS B, BUDDLE JR.** Proposed revisions to the serological typing system for *Treponema hyodysenteriae*. Epidemiol Infect 1989; 102: 75–84.
5. **COMBS BG, HAMPSON DJ, HARDERS SJ.** Typing of Australian isolates of *Treponema hyodysenteriae* by serology and by DNA restriction endonuclease analysis. Vet Microbiol 1992; 31: 273–285.
6. **MIAO RM, FIELDSTEEL AH.** Genetics of *Treponema*: characterization of *Treponema hyodysenteriae* and its relationship with *Treponema pallidum*. Infect Immun 1978; 22: 736–739.
7. **STANTON TB, JENSEN NS, CASEY TA, TORDOFF LA, DEWHIRST FE, PASTER BJ.** 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 1991; 41: 50–58.
8. **JENSEN NS, CASEY TA, STANTON TB.** Detection and identification of *Treponema hyodysenteriae* by using oligodeoxynuceotide probes complementary to 16S rDNA. J Clin Microbiol 1990; 28: 2717–2721.
9. **LEE JI, HAMPSON DJ, COMBS BG, LYMBERY AJ.** Genetic relationship between isolates of *Serpulina (Treponema) hyodysenteriae*, and comparison of methods for their subspecific differentiation. Vet Microbiol 1993; 34: 35–46.
10. **OWEN RJ.** Chromosomal DNA fingerprinting: a new method of species and strain identification applicable to microbial pathogens. J Med Microbiol 1989; 30: 89–99.
11. **HAREL J, COTE S, JACQUES M.** Restriction endonuclease analysis of porcine *Pasteurella multocida* isolates from Québec. Can J Vet Res 1990; 54: 422–426.
12. **STULL TL, LILIPUMA JJ, EDLIND TD.** A broadspectrum probe for molecular epidemiology of bacteria; ribosomal RNA. J Infect Dis 1988; 157: 280–286.
13. **GRIMONT F, GRIMONT PAD.** Ribosomal ribonucleic and gene restriction patterns as potential taxonomic tools. Ann Inst Pasteur Microbiol 1986; 137B: 165–175.
14. **GRIMONT F, GRIMONT PAD.** DNA fingerprinting. In: Stackebrandt E, Goodfellow M, eds. Nucleic Acid Techniques in Bacterial Systematics. New York: John Wiley & Sons, 1991: 249–279.
15. **BEAUDOIN M, HAREL J, HIGGINS R, GOTTSCHALK M, FRENETTE M, MacINNES JI.** Molecular analysis of isolates of *Streptococcus suis* serotype 2 by restriction-endonuclease-digested DNA separated on SDS-PAGE and by hybridization with an rDNA probe. J Gen Microbiol 1992; 138: 2639–2645.
16. **TER HUURNE AHM, VAN HOUTEN M, KOOPMAN MBH, VAN DER ZEIJST BAM, GAASTRA W.** Characterization of Dutch porcine *Serpulina (Treponema)* isolates by restriction endonuclease analysis and DNA hybridization. J Gen Microbiol 1992; 138: 1929–1934.
17. **JENSEN NS, CASEY TA, STANTON TB.** Characterization of *Serpulina (Treponema) hyodysenteriae* and related intestinal spirochetes by ribosomal RNA gene restriction patterns. FEMS Microbiol Lett 1992; 93: 235–242.
18. **Li Z, JENSEN NS, BELANGER M, L'ESPERANCE M-C, JACQUES M.** Molecular characterization of *Serpulina (Treponema) hyodysenteriae* isolates representing serotypes 8 and 9. J Clin Microbiol 1992; 30: 2941–2947.
19. **LEBLANC JD, LEE LN.** Rapid screening procedure for detection of plasmids in streptococci. J Bacteriol 1979; 140: 1112–1115.
20. **SAMBROOK J, FRISCHT EF, MANIATIS T.** Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1989.
21. **SAMONS DW, ADAMS LD, NISHIZAWA EE.** Ultrasensitive silver-based color staining of polypeptides in polyacrylamide gels. Electrophoresis 1981; 2: 135–141.
22. **BROSIUS J, ULLRICH A, RAKER MA, GRAY A, DULL JD, GUTELL RR, NOLLER HF.** Construction and fine mapping of recombinant plasmids containing the *rrnB* ribosomal operon of *E. coli*. Plasmids 1981; 6: 112–118.