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Characterization of Prototype and Clinically Defined Strains of *Streptococcus suis* by Genomic Fingerprinting

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A collection of *Streptococcus suis* strains from animal and human infections was examined for DNA-banding patterns after restriction endonuclease digestion and agarose gel electrophoresis. The endonuclease *Hae*III produced the most discriminating restriction profiles among 23 serotypes studied. DNA from serotypes 9, 11, 12, and 16 was resistant to *Hae*III cleavage. DNA from serotypes 9 through 16 was cleaved with *Hin*dIII and showed substantial genomic differences. We also examined 106 epidemiologically unrelated strains isolated from cases of pig meningitis or pneumonia and 5 strains isolated from cases of human meningitis in order to compare genomic fingerprinting and serotyping as epidemiological tools. Heterogeneity was found among fingerprints of serologically identical isolates, indicating genetic diversity within some serotypes. DNA fingerprints of some serotype 2 strains from different sources appeared identical, suggesting a clonal relationship among strains of this serotype. The data suggest that this technique represents an important tool for examining the natural history of disease caused by *S. suis*.

Streptococcus suis is an important emerging cause of meningitis, pneumonia, septicemia, and arthritis in young piglets (17, 18) and meningitis in humans (2, 6). Detailed epidemiologic studies are required to define the role of *S. suis* in these infections. However, the appropriate studies are hampered by a lack of sufficiently discriminatory typing systems to identify strains to the subspecies level.

Identification of *S. suis* is usually on the basis of morphology and biochemical or serologic reactions (12). The species constitutes a subgroup of Lancefield group D streptococci and is characterized by the presence of lipid-bound teichoic acid and capsular polysaccharide as antigens (9, 15). So far, 23 distinct serotypes have been described on the basis of serological reactions to the polysaccharide capsule (11, 15). The clonal relationship of different serotypes has not been studied. In addition, the association of a given serotype with specific pathogenic response is unclear.

Analysis of chromosomal DNA restriction endonuclease digestion patterns shows genomic rearrangements and profile differences among microorganisms of the same species. This approach has proven a valuable tool in epidemiological studies of other streptococcal species (8, 16). Among *Neisseria meningitidis* and group A streptococci this technique differentiated individual strains within serotypes (3, 7). Thus, chromosomal DNA fingerprints may provide a useful, alternative method for the characterization of *S. suis* strains.

This study reports the characterization of 23 serotypes of *S. suis* by restriction fragment length analysis of chromosomal DNA. To evaluate whether this method is useful in establishing differences among *S. suis* strains, we also analyzed isolates associated with meningitis or pneumonia, hoping to determine genetic relatedness within and among serotypes. The results described here suggest that this method represents an important tool for epidemiogical studies of *S. suis* in both swine and human infections.

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MATERIALS AND METHODS

Bacterial strains. *S. suis* strains were obtained from four different sources. Reference strains of 17 serotypes (serotypes 1/2 through 16) and four strains of *S. suis* isolated from abattoir workers in Hong Kong with a history of meningitis and one strain, serotype 4, isolated from a pigkeeper in the Netherlands with meningitis (2) were provided by the center for Reference and Research on Streptococci, University of Minnesota. Reference strains of serotypes 17 through 22 were obtained from the Faculté de Medicine Vétérinaire, Université de Montréal. Thirty-three strains of *S. suis* associated with outbreaks of swine meningitis were collected at the Animal Disease Research and Diagnostic Laboratory, South Dakota State University. Seventy-three isolates from cases of swine meningitis or pneumonia were collected at the Minnesota Veterinary Diagnostic Laboratory. The strains were grown in Todd-Hewitt broth and frozen at −70°C.

Serotyping. Serotyping was performed by the capillary precipitin, agglutination, and coagglutination methods with antisera prepared in rabbits. Some cross-reactions as indicated by double-gel immunodiffusion were removed by absorption (14).

DNA extraction and analysis. Total cellular DNA was extracted by using a modified method described by Gebhart et al. (10). Cells were grown overnight in 30 ml of Todd-Hewitt Broth with 1% yeast extract, recovered by centrifugation at 10,000 × *g*, and washed twice with phosphate-buffered saline. Cells were suspended in 5 ml of TE sucrose buffer (25% sucrose in 50 mM Tris hydrochloride [pH 8.0]–50 mM EDTA–10 mM NaCl) containing 10 mg of lysozyme per ml and incubated at room temperature for 60 min with frequent mixing. Proteinase K and Sarkosyl were added to final concentrations of 100 μg/ml and 1%, respectively. The resulting suspension was thoroughly mixed and incubated at 55°C for 3 h. Ammonium acetate was added to a final concentration of 2.5 M, and the tubes were kept on ice for 20 min. One hundred microliters of phenylmethylsulfonyl

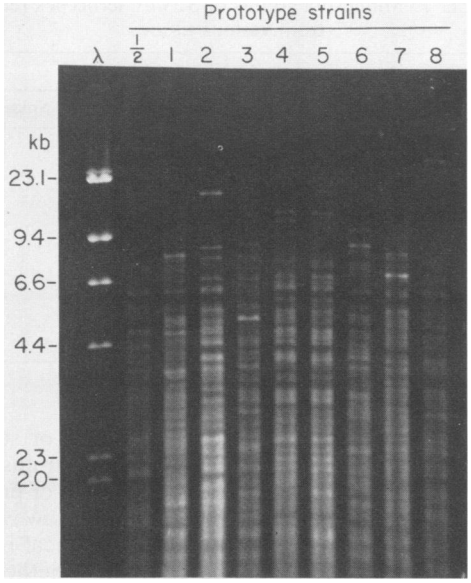

FIG. 1. DNA fingerprints of strains representing *S. suis* serotypes 1/2 through 8. Lane λ, Lambda *Hin*dIII size markers. kb, Kilobases.

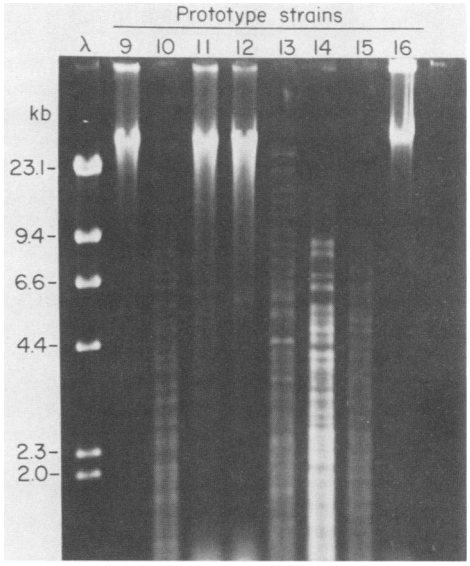

FIG. 2. DNA fingerprints of strains representing *S. suis* serotypes 9 through 16. Lane λ, Lambda *Hin*dIII size markers. kb, Kilobases.

fluoride was added (2 mg/ml stock in ethanol) to inactivate proteinase K, and the preparation was centrifuged at 10,000 × *g* for 20 min. DNA was precipitated from the supernatant with 2 volumes of cold absolute ethanol, washed with 70% ethanol, dried, dissolved in 500 μl of TE buffer, and stored at 4°C. The final DNA concentration was measured spectrophotometrically at 260 nm (13).

Restriction enzyme digestion and fractionation. Streptococcal DNA samples were digested for 2 h at 37°C in 30-μl volumes containing the restriction endonuclease *Hae*III or *Hin*dIII in appropriate buffers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Selected digests were repeated at least three times to establish the reproducibility of the method.

Methylation experiments. Two sets of isoschizomers were used to determine whether methylation was blocking the activity of *Hae*III digestion on DNA from serotypes 9, 11, 12, and 16. Isoschizomers *Msp*I and *Hpa*II were used to determine the extent of cytosine methylation at CCGG sites, and the isoschizomers *Sau*3AI and *Mbo*I were used to determine the presence of adenine methylation at GATC sites (13).

Agarose gel electrophoresis. Digested DNA was electrophoresed in a horizontal gel containing 0.55% agarose at 30 V for 17 h in Tris borate buffer (89 mM Tris base, 89 mM boric acid, and 2.4 mM sodium EDTA [pH 8.3]). DNA fragments were stained with 0.5 mg of ethidium bromide per ml for 1 h and then rinsed for 3 min in distilled water. DNA was visualized with shortwave UV light, and the gels were photographed with type 55 Polaroid film.

RESULTS

Analysis of *S. suis* DNA from prototype strains. It was found that *Hae*III and *Hin*dIII restriction endonucleases produced a convenient cleavage pattern of *S. suis* DNA. Of the two, *Hae*III provided the most discriminating pattern. Visual inspection of restriction profiles of serotypes 1/2 through 8 revealed clear genomic differences (Fig. 1). These were particularly evident in the larger fragments (4.4 to 23 kbp). However, purified DNA from serotypes 9, 11, 12, and 16 was reproducibly resistant to *Hae*III cleavage (Fig. 2).

In order to determine if the resistances of serotypes 9, 11, 12, and 16 to *Hae*III were caused by methylation of *S. suis* DNA, the activity of enzymes which were sensitive to cytosine or adenine modification was compared with that of enzymes which were insensitive to this modification. The *Hpa*II restriction enzyme does not cleave DNA when the internal cytosine is methylated, whereas *Msp*I is insensitive to cytosine methylation (13). DNAs from these strains were cleaved by isoschizomers *Msp*I and *Hpa*II (data not shown), and differences were detected in electrophoretic patterns. Likewise, *Mbo*I cleaves DNA if the adenine of the restriction site GATC is not methylated and *Sau*3AI is insensitive to methylation at the GATC site. Both enzymes digested DNAs from these serotypes (data not shown). Thus, resistance to *Hae*III digestion was not due to methylation of cytosine or adenine. The restriction profiles produced by these enzymes indicate that serotypes 9, 11, 12, and 16 did not represent a single clone.

To determine whether resistance to *Hae*III digestion was caused by a soluble inhibitor or contaminant contained in *S. suis* DNA preparations, a mixing experiment was performed. DNA from *S. suis* serotype 11 was separately mixed with lambda DNA before incubation with *Hae*III. Lambda DNA was digested despite the presence of *S. suis* DNA serotype 11, indicating that an inhibitor was not present (data not shown). Furthermore, DNA from serotypes 9, 11, 12, and 16 was readily digested by *Hin*dIII, demonstrating that inhibitors to this enzyme were not present. *Hin*dIII cleavage of serotypes 9, 11, 12, and 16 showed substantial genomic differences among the serotypes, which were also clearly distinguishable from serotypes 1/2 through 8 and 17 through 22 (Fig. 3). Clear differences in banding patterns were also discernable among prototype reference strains 17 through 22 when examined with *Hae*III (Fig. 4).

Restrictions analysis of pneumonic strains. Fifty-two of the 73 strains studied were isolated from diseased lungs, 12 were

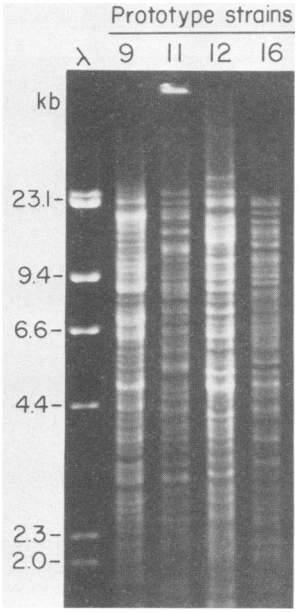

FIG. 3. DNA fingerprints of strains representing *S. suis* serotypes 9, 11, 12, and 16. Lane λ, Lambda *Hin*dIII size markers. kb, Kilobases.

from brains, 3 were from noses, 2 were from livers, 1 was from the spleen, and 3 were from hearts (Table 1). Because serotype 2 is the most commonly reported cause of disease, efforts were concentrated on 28 pneumonic strains which belonged to this serotype. The fingerprints of the strains were very heterogeneous. Representative patterns of some pneumonic strains (Fig. 5, lanes 7 to 10) differed from the prototype 2 strain (Fig. 5 lane 2) in two or four bands.

TABLE 1. Minnesota strains of *S. suis* serotypes recovered from various tissues

Serotype	No. of strains from:					
	Lung	Brain	Nose	Liver	Spleen	Heart
2	28	8	1	1		2
3	5	4				
4	7					
5	2					
7	1					
8	4		1	1	1	
9			1			
Nontypeable	5					1

The fingerprints of four meningeal strains of serotype 2 were compared with those of pneumonic strains (Fig. 5, lanes 3 to 6). As can be seen, the fingerprints of pneumonic and meningeal strains are clearly different. However, some epidemiologically unrelated strains had identical restriction profiles (data not shown). To determine whether genetic diversity existed in other serotypes, seven strains of serotype 4 including one from a case of human meningitis were examined. These strains also showed heterogeneity (data not shown). In addition, five nontypeable strains could be identified as belonging to serotype 2 when their profiles were compared with those of reference strains (data not shown).

Restriction endonuclease patterns of *S. suis* associated with meningitis. To explore the possibility that meningeal isolates were clonally related and different from normal flora or pneumonic strains, a variety of meningeal strains were compared. Examination of the genomic fingerprints of four strains from Hong Kong cases of human meningitis showed that they were similar but not identical to the prototype strain of serotype 2 (Fig. 6). Fingerprints shown in Fig. 6 in lane 3 (1984 isolate) and lane 5 (1987 isolate) were identical,

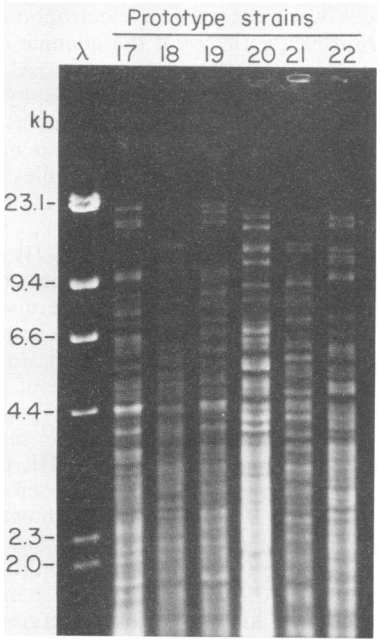

FIG. 4. DNA fingerprints of strains representing *S. suis* serotypes 17 through 22. Lane λ, Lambda *Hin*dIII size markers. kb, Kilobases.

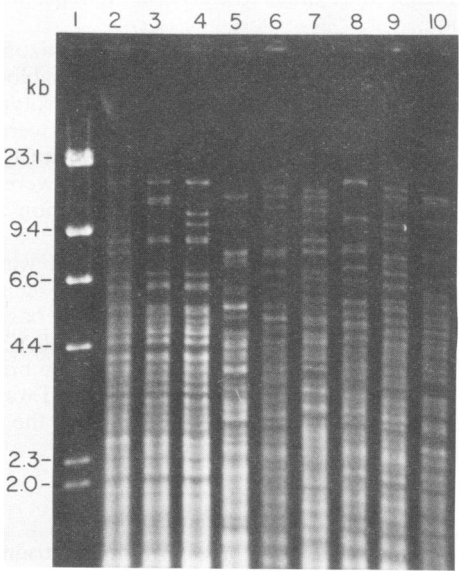

FIG. 5. Comparison of *Hae*III restriction profiles of serotype 2 strains associated with meningitis or pneumonia. Lane 1, Lambda DNA size markers; lane 2, prototype 2; lane 3, strain 18; lane 4, strain 29; lane 5, strain 25; lane 6, strain 27; lane 7, strain 123; lane 8, strain 109; lane 9, strain 75; lane 10, strain 80. kb, Kilobases.

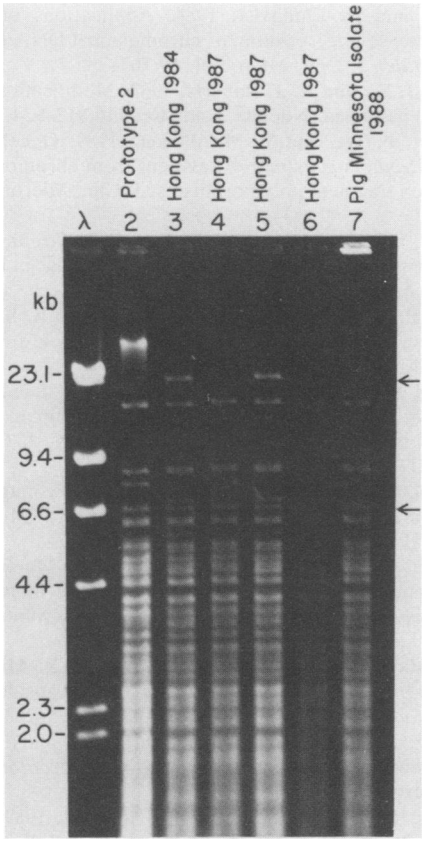

FIG. 6. Comparison of DNA fingerprints of *S. suis* type 2 strains isolated from cases of human meningitis. Arrows indicate unique fragments. Lane λ, Lambda DNA size markers; lane 2, prototype 2 (strains 86-130); lane 3, strain 84-551; lane 4, strain 87-553; lane 5, strain 87-554; lane 6, strain 87-555; lane 7, strain 88-025-5. The upper band in lane 2 corresponded to undigested DNA. kb, Kilobases.

but these patterns differed from that of the prototype 2 strain in two bands (arrows). Fingerprints in lanes 4 and 6 of Fig. 6 were also identical. Both DNAs represent two different 1987 isolates. The fingerprint of a serotype 2 strain isolated from a case of pig meningitis in Minnesota (Fig. 6, lane 7) was also examined; it had a profile identical to that of the two 1987 human isolates.

An additional 33 *S. suis* strains from randomly selected pig meningitis cases were also digested with *Hae*III. Four of these were serotype 1, and 29 were serotype 2. Serotype 1 isolates demonstrated a unique fragment pattern (data not shown). Twelve of 29 serotype 2 isolates had unique profiles; the remaining 17 isolates could be placed into one of five restriction profile groups (Table 2). Restriction profiles representative of the first two patterns of serotype 2 strains are

TABLE 2. South Dakota *S. suis* meningeal isolates with identical restriction profiles

Profile no.	ID[a] of isolates
1	18, 23, 26, 30
2	15, 17, 20, 24
3	8, 11, 27, 31
4	3, 5, 12
5	25, 2

[a] ID, Laboratory strain designation.

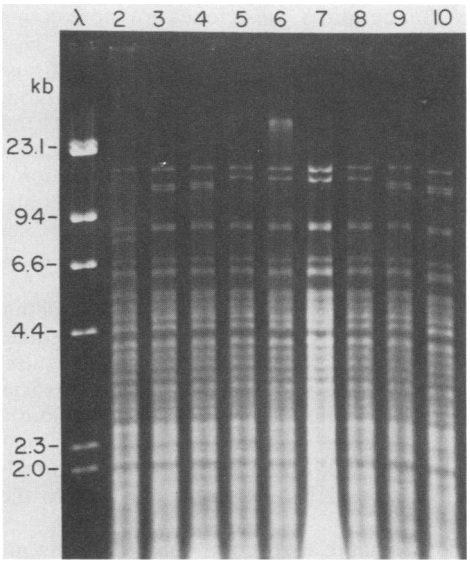

FIG. 7. Comparison of DNA fingerprints of pig meningeal isolates, serotype 2. Lane λ, Lambda DNA size markers; lane 2, prototype 2; lane 3, strain 18; lane 4, strain 23; lane 5, strain 24; lane 6, strain 15; lane 7, strain 17; lane 8, strain 20; lane 9, strain 26; lane 10, strain 30. kb, Kilobases.

shown in Fig. 7. DNAs shown in Fig. 7 in lanes 3, 4, 9, and 10 [pattern no. 1]) were identical to each other and also were similar to reference serotype 2 (Fig. 7, lane 2). Strains shown in Fig. 7 in lanes 5, 6, 7, and 8 (pattern no. 2) were identical to each other but differed by one or two fragments from the reference serotype 2 and from pattern no. 1 (Fig. 7).

DISCUSSION

The results of this study demonstrate that genomic fingerprinting can be used as a reliable tool to distinguish individual isolates of *S. suis*. This method has the potential to reveal information on the epidemiology of *S. suis* infections and will allow the examination of the transmission and origin of *S. suis* within a given population.

This technique has already been used successfully to compare strains from other streptococcal species (5, 7, 8, 16). In the present study restriction profiles produced by digesting chromosomal DNA with *Hae*III endonuclease proved very effective in discriminating among different isolates of *S. suis*. Substantial genotypic difference among serotypes was found. Although *Hae*III did not cut DNA from serotypes 9, 11, 12, and 16, *Msp*I, *Hpa*II, and *Hin*dIII cleavage yielded profiles which showed clear genomic differences among these prototype strains.

At this time there is no specific examination for the resistance to *Hae*III digestion of those four serotypes. It is possible that these strains of *S. suis* possess a restriction modification system specific for GGCC. Similar protective mechanisms have been described in other organisms, e.g., *Escherichia coli* and *Bacillus* and *Haemophilus* species (4).

When examining *S. suis* serotype 2 human isolates, it was found that they were similar but not identical to the prototype serotype 2 strain. Therefore, the technique permitted differentiation between isolates that were serologically indistinguishable. It is notable that isolates recovered from different patients at different points in time had identical genomic profiles. This indicates that a clonal relationship

exists and that certain *S. suis* clones are more commonly the cause of human meningitis, at least in the Hong Kong population. It is interesting that one meningitis pig isolate from Minnesota was identical to two Hong Kong human isolates obtained in 1987 (Fig. 6). This suggests that certain clones of *S. suis* may be widely dispersed and that pigs represent a potential source of human infection (6). This conclusion agrees with the results of Arends et al. (1), who did not find phenotypic differences between serotype 2 tonsillar isolates from carrier pigs and meningeal isolates from human patients.

Analysis of the restriction patterns of pig meningeal isolates revealed five different profiles within 17 isolates and 12 strains which had unique patterns. These results indicate that several different *S. suis* type 2 clones associated with meningitis exist among the pig population of South Dakota, but these isolates are, in fact, very similar. It appears from Fig. 7 that strains with profiles 1, 2, and 3 tend to be more common, because 12 isolates were placed into these three profiles.

On the other hand, restriction analysis of pig pneumonic strains showed a greater degree of heterogeneity. This genetic diversity, as evidenced by restriction polymorphisms in chromosomal DNA within a given serotype, is not unexpected. It has been observed in other organisms studied by using this technique (8, 16).

When the restriction patterns of meningeal and pneumonic isolates were compared, differences in banding patterns could be easily observed. However, some strains with identical restriction profiles were found. This observation suggests either a clonal association or the possibility that some have the capacity to cause either meningitis or pneumonia or both.

The results of this study indicate that restriction endonuclease analysis is a useful technique for characterizing individual strains of *S. suis* and when used in conjunction with serotyping in epidemiologic investigations provides a reliable, high-resolution method to assess the clonal relationship of strains isolated from different epidemiological or pathological settings.

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