

Virulent Strains of *Streptococcus suis* Serotype 2 and Highly Virulent Strains of *Streptococcus suis* Serotype 1 Can Be Recognized by a Unique Ribotype Profile

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The ribotype profiles of 42 different *Streptococcus suis* strains were studied. These strains belonged to five serotypes and differed in their virulence for pigs as well as in the expression of the muramidase-released protein and the extracellular protein factor. For the ribotyping, chromosomal DNAs were digested with *EcoRI* and were hybridized with a 1,066-bp ribosomal DNA probe. The hybridization patterns showed genetic heterogeneity within and between the serotypes. Pathogenic strains of serotype 2 and highly pathogenic strains of serotype 1 could be recognized by their unique ribotype profiles. Nonpathogenic strains showed a high degree of genetic heterogeneity. Moreover, by comparing the 16S ribosomal DNA sequences of a number of *S. suis* strains, we were able to design two DNA probes which specifically hybridized with *S. suis* strains.

Streptococcus suis is an important cause of meningitis, pneumoniae, septicemia, and arthritis in young pigs (6, 26) and can cause meningitis in humans (1). Attempts to control the disease are still hampered by the lack of knowledge about the epidemiology of the disease and the lack of effective vaccines and sensitive diagnostics.

S. suis strains are usually identified and classified by their morphological, biochemical, and serological characteristics (13, 14, 26). Serological classification is based on the presence of specific antigenic cell wall polysaccharides. At present, 35 different capsular types have been described (8, 9, 12, 19). In The Netherlands, as well as in other countries in Europe, *S. suis* serotype 2 is the most prevalent type isolated from diseased pigs, followed by serotypes 1 and 9. Strains of serotype 2, can differ in their virulence (25, 31). Pathogenicity in these strains is associated with the synthesis of two proteins, muramidase-released protein (MRP) and extracellular protein factor (EF) (28, 31). Three phenotypes can be distinguished: (i) strains which synthesize both MRP and EF (MRP⁺EF⁺) and which are pathogenic for young pigs, (ii) strains which synthesize MRP and enlarged forms of EF (MRP⁺EF*) and which are weakly pathogenic, and (iii) strains which synthesize neither MRP nor EF (MRP⁻EF⁻) and which are nonpathogenic for young pigs (28, 31).

Between and within the serotypes of *S. suis*, strains can be genetically heterogeneous. Because rRNA sequences in bacteria are highly conserved, analysis of strains by restriction fragment length polymorphisms of genes encoding rRNA (ribotyping) can provide valuable information for taxonomic, diagnostic, and epidemiological purposes (10, 18, 24, 33). Recent data indicate that ribotyping could be used for the detection of genetic differences in *S. suis* isolates (2, 17). These differences were not observed when serological or biochemical tests were used (2, 17).

The 16S rRNA sequence of reference strain 6555 of *S. suis*

serotype 1 has been partially determined (3). A comparative analysis of this sequence with the 16S rRNA sequences of 30 other species of the genus *Streptococcus* revealed that *S. suis* is a genetically distinct species which displays no specific relationship to other streptococcal species (3). Moreover, it was noted that in streptococcal species the highest variability is in the V1 and V2 regions of their 16S rRNAs (3, 4). The sequences of the V1 region of the 16S rRNAs of the other *S. suis* strains are yet to be determined.

In the present report we describe the results of the ribotyping of 42 different *S. suis* strains. These strains belonged to five serotypes and differed in their pathogenicities for pigs as well as in the production of MRP, EF, and EF*. Moreover, by comparing the 16S ribosomal DNA sequences of a number of *S. suis* strains, we were able to design two DNA probes which specifically hybridize with *S. suis*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *S. suis* reference strains were obtained from M. Gottschalk, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada. The various *S. suis* serotype 1, 1/2, 2, 4, and 9 strains were obtained from different sources (Table 1). Most strains were obtained from the Animal Health Services, Boxtel, Deventer, The Netherlands. These strains were isolated from the organs of diseased pigs. Other strains were isolated from the tonsils of healthy pigs after slaughter. Strain 6230 (serotype 4) was obtained from L. Galina, Department of Large Animal Clinical Sciences, University of Minnesota. The *Streptococcus pyogenes*, *Streptococcus uberis*, and *Streptococcus bovis* strains were from the laboratory collection of the DLO-Institute for Animal Science and Health.

The strains were grown in Todd-Hewitt broth (code CM189; Oxoid, Ltd., London, England) and were plated on Columbia agar blood base (code CM331; Oxoid) containing 6% horse blood.

Serotyping. The *S. suis* strains were serotyped by the slide agglutination test with serotype-specific antibodies (26).

Phenotyping. The strains were analyzed for the production of MRP and EF by Western blotting (immunoblotting) and enzyme-linked immunosorbent assays. For Western blot analysis proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15) with 4% stacking gels and 6% separating gels. The separated proteins were transferred to nitrocellulose filters in a semidry transfer cell (Bio-Rad Laboratories, Richmond, Calif.). To detect specific proteins the blots were incubated with monoclonal antibodies directed against MRP or EF (27). Bound antibodies were visualized with antirabbit sera conjugated with alkaline phosphatase (Zymed Laboratories, San Francisco, Calif.) as described by Sambrook et al. (20).

The presence of MRP, EF, and their variant proteins MRP*, MRP^s (a variant

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TABLE 1. Characteristics of *S. suis* strains

Strain	Serotype	MRP ^a	EF ^a	Source ^b	Virulence for pigs ^c
6388	1	s	+	O	HV
6290	1	s	+	CNS	HV
6112	1	s	+	O	HV
5637	1	—	—	T	AV
6555 Rd	1	—	—	U	V
5673	1/2	*	—	T	ND
5679	1/2	*	—	T	ND
5928	1/2	*	—	U	ND
5933	1/2	*	—	U	ND
5934	1/2	*	—	U	ND
5939	1/2	*	—	U	ND
6233	1/2	*	—	L	ND
6468	1/2	*	—	L	ND
2492	1/2	—	—	L	ND
5209 ^R	1/2	—	—	U	ND
5810	1/2	—	—	T	ND
3305	1/2	—	—	T	ND
D282	2	+	+	CNS	V
3	2	+	+	CNS	V
10	2	+	+	T	V
17	2	+	*	T	WV
24	2	+	*	H	WV
28	2	+	*	H	WV
T15	2	—	—	T	AV
12	2	—	—	T	AV
16	2	—	—	T	AV
5213 ^R	4	s	—	T	ND
5639	4	s	—	U	ND
6080	4	s	—	L	ND
6145	4	s	—	L	ND
5997	4	s	—	U	ND
6136	4	s	—	U	ND
5638	4	—	—	U	ND
5921	4	—	—	T	ND
5920	4	—	—	U	ND
6230	4	—	—	O	ND
6473	4	—	—	CNS	ND
6072	4	—	—	L	ND
5973	9	*	—	CNS	AV
6437	9	*	—	O	AV
5218 ^R	9	—	—	U	AV
6207	9	—	—	T	AV

^a +, protein is present; —, protein is absent; *, variant of protein with a higher molecular mass is present; s, protein with a lower molecular mass is present.

^b Strains were derived from pigs unless they are designated with H or U. CNS, central nervous system; T, tonsils; H, human; L, lungs; O, various organs; U, source unknown.

^c HV, highly virulent; V, virulent; WV, weakly virulent; AV, avirulent; ND, not determined (23, 28, 31).

^d R, reference strain.

of MRP with a lower molecular mass), and EF* in culture supernatants of *S. suis* cultures was determined with MRP- and EF-specific double-antibody sandwich enzyme-linked immunosorbent assays as described by Vecht et al. (27).

Pathogenicity. The pathogenicities of the *S. suis* strains were determined in germfree pigs, as described before (28). Briefly, 5-day-old pigs were inoculated intranasally with about 10⁷ CFU of *Bordetella bronchiseptica* 92932. Two days later the pigs were inoculated intranasally with *S. suis* (10⁶ CFU for *S. suis* serotypes 2 and 9 and 10⁴ CFU for *S. suis* serotype 1). Pigs were monitored twice daily for clinical signs of disease. Blood samples were collected three times a week from each pig. The numbers of leukocytes were counted with a cell counter. To monitor infection with *S. suis* and *B. bronchiseptica*, we collected nasopharyngeal swab specimens and feces daily. The swabs were plated directly onto Columbia agar containing 6% horse blood. After the pigs were killed, they were examined for pathological changes. Tissue specimens from the central nervous

system, serosae, and joints were examined bacteriologically and histologically as described previously (28).

Ribotyping. Chromosomal DNA was isolated and digested with restriction enzymes as described by Sambrook et al. (20). DNA fragments were separated on 0.8% agarose gels, and the fragments were transferred to Zeta-Probe GT membranes (Bio-Rad Laboratories) as described by Sambrook et al. (20). We used two different hybridization procedures. (i) The DNA on the blots was hybridized overnight at 65°C with a 1,066-bp 16S ribosomal DNA probe as recommended by the supplier of the Zeta-Probe membranes. After hybridization, the membranes were washed twice with a solution of 40 mM sodium phosphate (pH 7.2), 1 mM EDTA, and 5% sodium dodecyl sulfate for 30 min at 65°C and twice with a solution of 40 mM sodium phosphate (pH 7.2), 1 mM EDTA, and 1% sodium dodecyl sulfate for 30 min at 65°C.

(ii) The DNA on the blots was hybridized overnight at 42°C with oligonucleotides specific for 16S rRNA domains V1 and V2 (see below) in a solution of 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]), 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 2% sodium dodecyl sulfate, and 100 μg of sheared salmon sperm DNA per ml. After hybridization, the membranes were washed twice for 30 min each time at 42°C in a solution of 3× SSC, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, and 5% sodium dodecyl sulfate.

Probes. As probes we used a 1,066-bp fragment from the 16S ribosomal DNA region. To obtain this fragment, the chromosomal DNA of strain 10 was used as a template in a PCR with the oligonucleotides 5'-GGTTGCGCTCGTTGCGG GAC-3' and 5'-GAGAGTTTGTATCCTGGCTCAGGA-3'. These oligonucleotides are located at positions 1113 to 1094 and 6 to 28, respectively (numbering according to that used for *Escherichia coli* [5]) of the 16S rRNA sequence of *S. suis* (3). The PCR product was labeled with [α-³²P]dCTP (3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) with a randomly primed labeling kit (Boehringer, Mannheim, Germany). In addition, two oligonucleotides were used as probes. The oligonucleotide 5'-TGAAGTATCGTGCTTACTAGACGG ATG-3' is specific for the V1 region of the 16S rRNA sequence of *S. suis* and corresponds to positions 70 to 98 (*E. coli* numbering [5]). The oligonucleotide 5'-TAACAGTATTTACCGCATGGTAGATA-3' is specific for the V2 region of the 16S rRNA sequence of *S. suis* and corresponds to positions 180 to 205 (*E. coli* numbering [5]). The oligonucleotides were labeled with [γ-³²P]dATP (5,000 Ci/mmol; Amersham Corp.) as described by Sambrook et al. (20).

DNA sequence analysis. The 16S ribosomal DNA fragments were amplified in a PCR by using chromosomal DNAs of various *S. suis* strains as templates. In these reactions we used the primers 5'-GAGAGTTTGTATCCTGGCTCAGGA -3' (positions 6 to 28; *E. coli* numbering [5]) and 5'-GGTTGCGCTCGTTGCGG GGAC-3' (positions 1113 to 1094; *E. coli* numbering [5]). The PCR products were purified by use of a QIAquick-spin PCR purification kit (Qiagen Inc., Chatsworth, Calif.), and the purified products were used for sequence analysis. In this analysis the primers 5'-GATCACCTCTCAGGTAGGC-3' (positions 289 to 308; *E. coli* numbering [5]) and 5'-GAGAGTTTGTATCCTGGCTCAGGA-3' (positions 6 to 28; *E. coli* numbering [5]) were used. DNA sequences were determined on a 373A DNA Sequencing System (Applied Biosystems, Foster City, Calif.). Samples were prepared by use of an ABI/PRISM dye terminator cycle sequencing kit (Perkin-Elmer, Foster City, Calif.).

RESULTS

Phenotypes and pathogenicity. We measured the expression of MRP and EF in a number of *S. suis* strains belonging to serotypes 1, 1/2, 4, and 9. Previously, we described that within serotype 2 three phenotypically different strains (MRP⁺EF⁺, MRP⁺EF*, and MRP⁻EF⁻) could be distinguished (28, 31) (Table 1). After serotype 2, serotypes 1 and 9 are the most prevalent types isolated from diseased pigs. Within *S. suis* serotype 1, two phenotypically different strains could be distinguished (Table 1). Strains showing the first phenotype (MRP^sEF⁺) produced the 110-kDa EF and a protein of about 120 kDa which is highly homologous with MRP but which has a lower molecular mass (29, 30). This smaller protein was designated MRP^s. Previously, we showed that the 136-kDa MRP contains a number of amino acid repeats (21). Analysis of the genes encoding MRP and MRP^s by PCR and Southern hybridization revealed that the differences in molecular mass between MRP and MRP^s resulted from differences in the number of amino acid repeats (22). Strains showing the second phenotype (MRP⁻EF⁻) produced neither MRP nor EF. The MRP^sEF⁺ strains of serotype 1 were highly pathogenic for young germfree pigs and caused septicemia and acute death within 48 h after intranasal inoculation (23). MRP⁻EF⁻ strain 6555 of serotype 1 was much less virulent, but it still induced

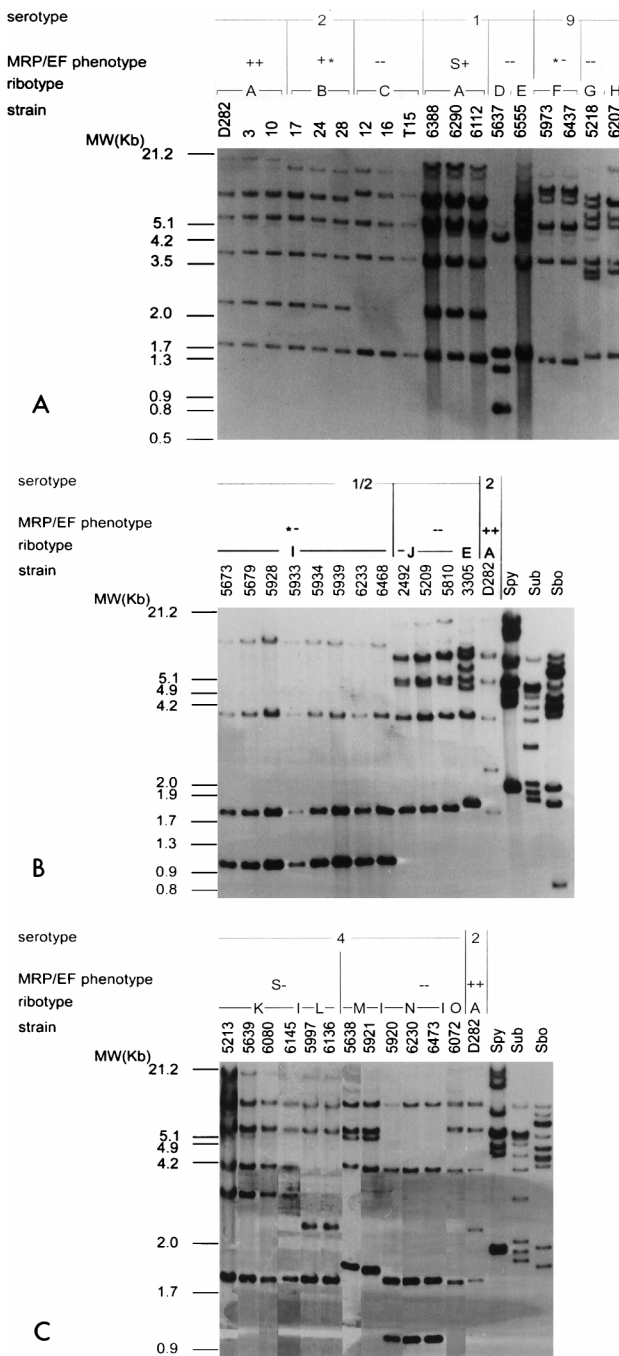


FIG. 1. Southern blots of chromosomal DNAs of various *S. suis* strains. Chromosomal DNAs were digested with *EcoRI* and probed with a 1,066-bp ribosomal DNA fragment. (A) Chromosomal DNAs of strains of serotypes 2, 1, and 9. (B) Chromosomal DNAs of strains of serotypes 1/2 and 2 as well as chromosomal DNAs of *S. pyogenes* (Spy), *S. uberis* (Sub) and *S. bovis* (Sbo) strains. (C) Chromosomal DNAs of strains of serotypes 4 and 2 as well as chromosomal DNAs of *S. pyogenes* (Spy), *S. uberis* (Sub), and *S. bovis* (Sbo) strains. Strain designations are indicated above the lanes. The serotypes of the strains, their phenotypes (synthesis of MRP, EF, and variants thereof), and their ribotype profiles are indicated above the lanes. The molecular size markers (MW) are indicated (in kilobases).

meningitis, polyarthritis, and occasionally, death 7 to 10 days after inoculation. However, MRP⁻EF⁻ strain 5637 did not induce any signs of disease (23).

Within *S. suis* serotype 9 we could also distinguish two phe-

notypically different strains (29, 30). Strains of the first phenotype (MRP⁺EF⁻) produced MRP-like proteins with molecular masses of about 148 kDa. These strains did not produce EF. Compared with the 136-kDa MRP, MRP⁺ contained more amino acid repeats (22). Strains of the second type (MRP⁻EF⁻) produced neither MRP nor EF. The MRP⁺EF⁻ as well as the MRP⁻EF⁻ strains of serotype 9 proved to be nonpathogenic for young germfree pigs after intranasal inoculation (29). After intravenous inoculation, however, the MRP⁺EF⁻ strains could induce some signs of disease (29).

Strains of serotype 1/2 belonged either to an MRP⁺EF⁻ phenotype (molecular mass of MRP⁺, 140 kDa) or to an MRP⁻EF⁻ phenotype. Strains of *S. suis* serotype 4 had either an MRP⁻EF⁻ or an MRP⁺EF⁻ phenotype. The MRP⁺EF⁻ strains produced an MRP of about 125 kDa (30). The pathogenicity of the serotype 1/2 and 4 strains for pigs has not been tested so far.

Ribotypes. To determine the ribotype patterns of the various *S. suis* strains, Southern blots of *EcoRI*-digested chromosomal DNAs were hybridized with a 1,066-bp 16S ribosomal DNA probe. The hybridization patterns showed genetic heterogeneity within and between the *S. suis* serotypes. Among the 42 strains analyzed, 15 different profiles were identified (Fig. 1; Table 2). Surprisingly, the pathogenic MRP⁺EF⁺ strains of serotype 2 and the highly pathogenic MRP⁺EF⁺ strains of serotype 1 had identical profiles (profile A). Therefore, this profile seemed to be specific for pathogenic strains producing both MRP (or MRP⁺) and EF. The weakly pathogenic MRP⁺EF⁺ strains of serotype 2 showed similar profiles as well (profile B). Profiles A and B looked very much alike, but they differed in their high-molecular-mass bands.

All eight strains of serotype 1/2 with the MRP⁺EF⁻ phenotype showed identical profiles (profile I). However, this differed from the profile of the two MRP⁺EF⁻ strains of serotype 9 (profile F). Among the six MRP⁺EF⁻ strains of serotype 4, two different profiles (profiles K and L) were identified. Strains with an MRP⁻EF⁻ phenotype were very heterogeneous. Among the 17 strains showing this phenotype, nine different profiles were observed. The profiles of the two MRP⁻EF⁻ strains of serotype 1 differed from each other (profiles D and E). However, profile E was shared with one of the MRP⁻EF⁻ strains of serotype 1/2.

16S rRNA sequences. We determined the nucleotide sequences of the V1 and V2 regions of the 16S ribosomal DNAs of a number of *S. suis* strains belonging to serotypes 1, 2, and 9 and having different phenotypes and different ribotype patterns. The nucleotide sequence of the 5' region of the 16S ribosomal DNA of strain D282 (serotype 2, MRP⁺EF⁺) is shown in Fig. 2. Analysis of the nucleotide sequences revealed that the V1 and V2 regions of the various 16S rDNA sequences were almost identical (data not shown). Moreover, the sequence of the V2 region was identical to the sequence described by Bentley et al. (3). These data confirm that the 42 strains examined belong to the species of *S. suis*. Because streptococcal species show high variabilities in the V1 and V2 regions of their 16S rRNAs, these regions could prove suitable for use in designing DNA probes specific for *S. suis* strains. Two oligonucleotides were selected and tested as probes on *EcoRI* digests of chromosomal DNAs of various *S. suis* strains and of an *S. uberis*, an *S. bovis*, and an *S. pyogenes* strain. Figure 3 shows that both probes discriminated between the *S. suis* strains and the other streptococcal species. Therefore, these probes may prove to be useful for identifying *S. suis* strains both for diagnostic purposes and in epidemiological studies.

TABLE 2. Ribotype profiles

Serotype	Profile for strains with the following pathogenicity and phenotype ^a								
	V			WV, MRP ⁺ EF*	AV		ND		
	HV, MRP ⁺ EF ⁺	MRP ⁺ EF ⁺	MRP ⁻ EF ⁻		MRP ⁺ EF ⁻	MRP ⁻ EF ⁻	MRP ⁺ EF ⁻	MRP ⁺ EF ⁻	MRP ⁻ EF ⁻
1	A ³		E ¹			D ¹			
2		A ³		B ³		C ³			
9						G ¹ ,H ¹			
1/2								I ⁸	
4									K ⁴ ,L ² E ¹ ,J ³ M ² ,N ³ ,O ¹

^a Different profiles are indicated with different capital letters. The superscript numbers indicate the number of strains analyzed. HV, highly virulent; V, virulent; WV, weakly virulent; AV, avirulent; ND, not determined.

DISCUSSION

The use of restriction fragment length polymorphism analysis for genes encoding rRNA (ribotyping) is an established technique for studying relationships between microorganisms (10, 18, 24, 33). Usually, it allows for the detection of different strains of the same species (3, 10, 18, 24, 33). Therefore, this method can be very useful for epidemiological and transmission studies. Recently, this technique was shown to be successful in detecting genetic differences in *S. suis* strains that could not be observed by serological and biochemical tests (2, 11, 17). The restriction endonuclease patterns of total chromosomal DNA has also been used to discriminate between *S. suis* strains (16, 17). However, these patterns contained an abundance of DNA bands which did not allow reliable comparisons and conclusions to be made (17).

We previously described that two proteins, MRP (or MRP^s) and EF, are associated with pathogenic strains of *S. suis* type 2 as well as with highly pathogenic strains of *S. suis* type 1. In the present work we showed that strains which synthesize both MRP (or MRP^s) and EF had a unique ribotype profile (profile A). This profile was independent of the serotype of the strains. These data suggest that these strains are very closely related and may have originated from a common ancestor. Moreover, these data suggest that ribotyping can be used to detect highly pathogenic *S. suis* type 1 and pathogenic *S. suis* type 2 strains. Compared with the highly pathogenic strains of serotype 1, the reference strain 6555 had a reduced pathogenicity, but could still cause disease in pigs (23). This strain did not synthesize MRP or EF and had a different ribotype profile (profile E). Profile E was also associated with one of the MRP⁻EF⁻ strains of serotype 1/2. The pathogenicity of this serotype 1/2 strain is as yet unknown.

Profile B seemed to be specific for MRP⁺EF* strains of serotype 2. Profiles A and B looked very much alike, differing only in the high-molecular-mass bands. MRP⁺EF* strains were only weakly pathogenic for young pigs. Strains with an MRP⁺EF* phenotype were predominantly isolated from human patients (26), suggesting that these strains are more

pathogenic for humans than for pigs. In humans the portals of entry of *S. suis* are probably small infected wounds (1); in pigs these are most probably the mucosae of the nasopharynxes and the palatine tonsils (7, 32). Therefore, the early pathogenesis of *S. suis* infections in pigs and humans may differ.

Recently, Okwumabua et al. (17) described that nonpathogenic strains of *S. suis* serotype 2 could be distinguished from pathogenic strains by ribotyping. In those studies, two nonpathogenic strains (strains DH5 and 0891) and one pathogenic strain (strain 86-3977B) were used. We compared the published profiles of these strains with our ribotype profiles. The profile of virulent strain 86-3977B seemed to be identical to profile A, which was the profile of the MRP (or MRP^s)- and

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1  AATACATGCA AGTAGAACGC TGAAGTCTGG TGCTTGCAC T AGACGGATGA GTTCGGAACG
61  GGTGAGTAAC GCGTAGGTAAC CCTGCCTCAT AGCGGGGGAT AACTATTGGA AACGATAGCT
121 AATACCGCAT AACAGTATTT ACCGCATGGT AGATATTTGA AAGGAGCBAU TGCTTCACTA
241 TGAGATGGAC CTGCGTTGTA TTAGCTAGTT GGTGAGGTAA CGGCTCACCA AGGCTTCGAT
361 ACATAGCCGA CCTGAGAGGG TGATCGGCCA CACTGGGACT GAGACACGGC CAGACCTCCT
481 ACGGGAGGCA GCAGTAGGGA ATCTTCGGCA ATGGGGGCAA CCCTGACCGA GCAACGCCGC
601 GTGAGTGAAG AAGGTTTTTCG GATCGTAAAG CTCTGTTGTA AGAGAAGAAC TGTGAGAAGA
721 GTGGAAGATT TCTCACTTGN CGGTATCATA CCAGATAGGG GCGGCTACCC ACGTGCACGC
841 ACCCTCGGTA AAACGTAGGT CCGGAGCGTT GTCCGGATTT ATTGGGCGTA AAGCGAGCGC
961 AGGCGGTTTG ATAGTCTGTA AGCAAAAGGC TGTGGCCTAA CCTAGTACG CTTTGGAAAC
1081 TGNCAAACCTT GAGTCCAGAA GGGGAGAGTG GAATTCATG GTAGCGGTGA AATNCGTAGA
1201 TATATGGAGG AACACCGGTC GCGAAAGCCG CTCTCTGGTC TGTAACTGAC GCTGAGGCTC
1321 GAAAGCGTGG GG
    
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FIG. 2. Nucleotide sequence of the 5' end of the ribosomal DNA region of *S. suis* D282 (serotype 2; MRP⁺EF⁺). The V1 and V2 regions are underlined.

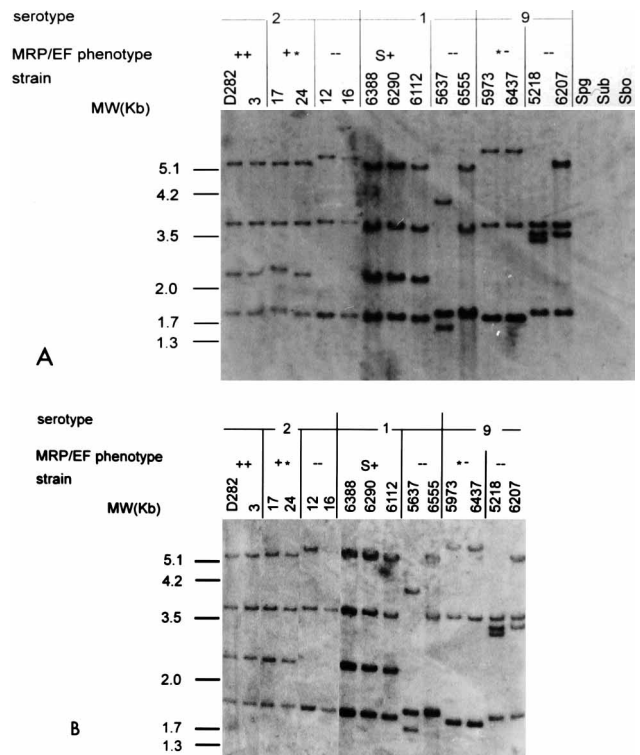


FIG. 3. Southern blots of chromosomal DNAs of various *S. suis* strains and *S. pyogenes* (Spy), *S. uberis* (Sub), and *S. bovis* (Sbo) strains. The chromosomal DNAs were digested with *EcoRI* and probed with a DNA probe specific for the V1 region of the 16S ribosomal DNA (A) and a DNA probe specific for the V2 region of the 16S ribosomal DNA (B). Strain designations are indicated above the lanes. The serotypes of the strains, their phenotypes (production of MRP and EF), and their ribotype profiles are indicated above the lanes. The molecular size markers (MW) are indicated (in kilobases).

EF-positive strains. The profiles of nonpathogenic strains DH5 and 0891 seemed similar to profile I (MRP⁺EF⁻ strains) except for an extra band of about 5 kb. Overall, these data support our observation that ribotype profile A is associated with pathogenic serotype 2 strains. If it is the case that pathogenic and nonpathogenic serotype 2 strains can be distinguished by their ribotype patterns, the method may prove to be important in diagnosing *S. suis* serotype 2 infections and in controlling the disease. Moreover, although not all pathogenic *S. suis* serotype 1 strains will be detected by ribotyping, the method could be used for the detection of the highly pathogenic strains. In addition, the method can contribute to our knowledge concerning the transmission and epidemiology of diseases caused by *S. suis*.

The data presented here, as well as those presented previously (17), also indicate that a considerable genetic heterogeneity exists between and within *S. suis* serotypes and phenotypes. The highest variability was observed between strains with an MRP⁻EF⁻ phenotype. Since we consider MRP⁻EF⁻ strains to be nonpathogenic, these data are in accordance with the results obtained by Beaudoin et al. (2), who showed that isolates from clinically healthy pigs showed more heterogeneous restriction enzyme profiles than those from diseased pigs.

Despite the heterogeneity in ribotype profiles, the sequences of the V1 and V2 regions of the 16S ribosomal DNAs of a number of *S. suis* strains were almost identical. On the basis of these data we designed oligonucleotide probes complementary to the V1 and V2 regions. Both probes specifically hybridized to DNAs from *S. suis* strains. Therefore, these probes may be useful as diagnostic tools in the future identification of *S. suis* strains. However, since only a limited number of streptococcal species was tested, a more comprehensive study is necessary to determine the specificities of these probes.

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