Genomic Relatedness among Reference Strains of Different *Streptococcus suis* Serotypes

Josée Harel, Robert Higgins, Marcelo Gottschalk and Michel Bigras-Poulin

ABSTRACT

Hybridization studies using genomic DNA and a rDNA probe revealed genetic relatedness among reference strains of different *Streptococcus suis* serotypes. Although most serotype 22 isolates are biochemically atypical, the reference strain of capsular type 22 is genetically related to other *S. suis* serotypes, but not to *Streptococcus pneumoniae*. Using DNA digested with *Bam*HI and *Bgl*II for ribotyping, some *S. suis* reference strains had common patterns, but this analysis mainly revealed variations in patterns of *S. suis* strains of different serotypes.

RÉSUMÉ

Des études d'hybridation utilisant des sondes d'ADN génomiques et d'ADNr révèlent une certaine similitude génétique parmi les souches de référence de *Streptococcus suis* représentant divers sérotypes. Quoique plusieurs isolats de *S. suis* sérotype 22 sont biochimiquement atypiques, la souche de référence du sérotype 22 semble être génétiquement apparentée aux souches de *S. suis* représentant d'autres sérotypes mais non à *Streptococcus pneumoniae*. Certaines souches de référence de *S. suis* présentent des patrons de ribotypes similaires lorsque l'ADN génomique digéré par *Bam*HI ou *Bgl*II est hybridé avec une sonde d'ADNr. Cependant, des variations dans le patron des ribotypes de souches de référence de *S. suis* ont été observées.

INTRODUCTION

Streptococcus suis is an important pathogen of swine. This microorganism causes septicemia, meningitis, arthritis, and pneumonia (1). It has also been associated with various pathologies in ruminants (2) and humans (1, 3). To date, 29 different capsular types of *S. suis* have been described (4–6). *Streptococcus suis* capsular type 2 is thought to be the most virulent serotype and, in most countries, it is the most prevalent type isolated from diseased animals (7–9).

Previous chemotaxonomic and deoxyribonucleic homology studies had indicated that *S. suis* strains serotypes 1 to 8 and 1/2 were highly related regardless of the capsular type (10). The DNA homology of different strains averaged more than 80%, with a G+C percentage of 40.7 to 38 mol % and it was concluded that these strains belonged to one species (10). Nonetheless, we and others have observed genotypic diversity among *S. suis* capsular type 2 isolates (11–13), similar to strains of *Streptococcus zooepidermicus* (14). Clear genomic differences in *Hae*III restriction enzyme profiles of strains of serotypes 1 through 8 and 1/2 and among *S. suis* reference strains of different capsular types (capsular types: 9, 11–15, 17–26) have been demonstrated (13). All *S. suis* reference strains have similar basic biochemical profiles, with the exception of capsular type 22 isolates, which are atypical when a large battery of tests are used (15).

The rRNA operons (including 5S, 16S, 23S and some tRNA genes) are present in several copies in the bacterial chromosome (16). Therefore, the use of an rRNA probe for comparative purposes is possible, as demonstrated in studies with other microorganisms (17, 18). With this technique, the electrophoretic pattern which contains numerous restriction fragments is easy to read. Ribotyping has also been used to study the distribution of strains within different bacterial species (19–22). The homogeneity found by ribotyping *S. suis* capsular type 2 isolates suggests that the isolates of one serotype are genetically similar (11).

The purpose of this study was to determine the genetic relatedness of reference strains of *S. suis* belonging to different serotypes, in order to determine intraspecies variations by examining rRNA gene restriction patterns and by hybridization analysis using *S. suis* chromosomal probes.

MATERIALS AND METHODS

BACTERIAL STRAINS

The reference strains of *S. suis* capsular types 1/2, 2–5, 9, 11–15, 17–26 were used (Table I). These strains originated from our collection (4–6, 15). Strains of *Streptococcus pneumoniae* (serotype 5, Ambrose from Dr. J. Henrichsen, Statens Seruminstitut, Copenhagen), *Streptococcus bovis* (79–1582) and *Escherichia coli* (P80-4787) were used as controls. Strains were cultured on trypticase soy agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Maryland) with 5% bovine blood at 37°C for 18 h. Stock cultures were stored at −70°C with 5% (w/v) of Dextran T40 (Pharmacia Biotech Inc., Baie d'Urfé, Québec) and 7% (w/v) of sucrose. An *E. coli* strain containing plasmid pKK3535 was kindly provided by Dr. J. Brosius, Harvard University, Boston, Massachusetts (23).

Groupe de Recherche sur les Maladies Infectieuses du Porc, Département de pathologie et microbiologie, Faculté de Médecine vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe, Québec J2S 7C6.

Submitted July 5, 1993.

TABLE I. Description and origin of *Streptococcus suis* reference strains used in this study

Strain	Serotype	Origin	References
5428	1	The Netherlands	(4)
2651	1/2	The Netherlands	(4)
735	2	The Netherlands	(4)
4961	3	Denmark	(4)
6407	4	Denmark	(4)
11538	5	Denmark	(4)
2524	6	Denmark	(4)
8074	7	Denmark	(4)
14636	8	Denmark	(4)
22083	9	Denmark	(15)
4417	10	Denmark	(15)
12814	11	Denmark	(15)
8830	12	Denmark	(15)
10581	13	Denmark	(15)
13730	14	The Netherlands	(15)
NCTC10446	15	The Netherlands	(15)
2726	16	Denmark	(15)
93	17	Canada	(15)
NT77	18	Canada	(15)
42A	19	Canada	(15)
86-5192	20	United States	(15)
14A	21	Canada	(15)
88-1861	22	Canada	(15)
89-2479	23	Canada	(6)
88-5299A	24	Canada	(6)
89-3576-3	25	Canada	(6)
89-4109-1	26	Canada	(6)

ISOLATION OF DNA, RESTRICTION ENDONUCLEASE DIGESTS AND SOUTHERN BLOTTING

Total cellular DNA was isolated using a modification of the method of Leblanc and Lee (24) described previously (11). DNAs were digested separately using a fourfold excess of either *Bam*HI or *Bgl*II according to the manufacturer's instructions (Pharmacia Biotech Inc.) Restriction fragments were separated by electrophoresis 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. After electrophoresis, DNAs were transferred to a nylon membrane (Zeta-Probe Membranes, Bio-Rad Laboratories, Richmond, California) for 18 to 24 h; the blots were hybridized overnight and subjected to a stringent wash in 0.2 × SSC at 50°C as described (25). A 7.7 kb fragment containing the *rrn*B operon encoding tRNAglu, 5S, 16S and 23S RNA and lambda rif^d, sequence genes was isolated by gel purification from pKK3535 (26) and labelled by random priming (Pharmacia Biotech Inc.) (11).

DNA SLOT BLOTTING HYBRIDIZATION

Five hundred ng of bacterial genomic DNA in 100 µL were applied onto Zeta-probe filters (Bio-Rad Laboratories) using a microfiltration apparatus (Bio-Rad Laboratories). For hybridization, the DNA was denatured by alkali treatment (100°C for 10 min in NaOH 0.4 M, EDTA 10 mM), neutralized with an equal volume of 2 M of cold ammonium acetate pH 7.0, and filtered on a Zeta-Probe membrane (Bio-Rad Laboratories) using a Bio-Dot SF blotting apparatus (Bio-Rad Laboratories). The blots were hybridized overnight and subjected twice to a stringent wash in: 1 mM EDTA, 40 mM $NaPO_4$, pH 7.2, 5% SDS at 65°C; and twice in 1 mM EDTA, 40 mM $NaHPO_4$, pH 7.2, 1 % SDS at 65°C.

Total cellular DNAs of *S. suis* serotypes 2 and 22 and of *S. pneumoniae* were labelled in vitro by ^{32}P random priming (26). Labelled probes were added to the prehybridization mixture and the incubation was continued for 16 h. Both prehybridization and hybridization were performed at 65°C under agitation. Hybridization signals were detected by autoradiography using X-OMAT AR films (Eastman Kodak Co., Rochester, New York) exposed overnight at −70°C with intensifying screens (Cronex Lightening-PlusTM, DuPont de Nemours & Co., Wilmington, Delaware).

RESULTS

GENOMIC PROBE HYBRIDIZATION

Genomic DNA probes from *S. suis* serotypes 2 and 22 hybridized with the DNA of reference strains of all tested serotypes but not with DNA from *S. pneumoniae* or *E. coli* (Fig.1). This observation confirms the relatedness among the serotypes of *S. suis*. A weak hybridization signal was observed with probes from reference strains *S. suis* serotypes 2 and 22 against *S. bovis*, which was less intense than hybridization with other *S. suis* reference strains. The hybridization signals using genomic probe *S. suis* serotype 2, with strains of *S. suis* of serotypes 8, 13 and 25, were less intense than hybridization with other *S. suis* reference strains. Moreover, the hybridization signals using genomic probe *S. suis* serotype 22, with strains of *S. suis* of serotypes 4, 5, 8, 9, 12, 13, 16 and 25, were less intense than hybridization with other *S. suis* reference strains but stronger than hybridization with *E . coli* or *S. pneumoniae*. This might be indicative of the degree of hybridization between *S. suis* reference strains. Although a weak signal was observed with reference strain of *S. suis* serotype 18, genomic DNA probe from *S. pneumoniae* did not hybridize with the tested serotypes of *S. suis*, *S. bovis* or *E. coli*, but hybridized with DNA from *S. pneumoniae*.

RIBOSOMAL PROBE HYBRIDIZATION

The DNA-rDNA hybridization analysis was performed on all *S. suis* strains using an *E. coli* rDNA derived from plasmid pKK3535. *Bam*HI and *Bgl*II restriction endonucleases produced a convenient cleavage pattern and ribotypes of *S. suis* DNA. Ribotype profiles of the different capsular types revealed clear genomic differences. Common bands were seen with *Bam*HI in the region of 21, 14, 12 kb (Fig. 2A), nine major ribotypes patterns could be observed. Based on their pattern of *Bam*HI bands, many reference strains showed similar patterns: serotypes 2, 14, 15 had similar patterns and serotypes 1/2, 3, 4, 5, 9, 23 had similar patterns. Common bands were seen with *Bgl*II in the region of 21, 13, 10.5 and 2.9 kb (Fig. 2B). More heterogeneity was observed when DNAs were digested with *Bgl*II. In this case, 12 major ribotypes patterns could be observed. Serotypes 2, 3, and 14 had similar patterns and serotypes 4, 5 and 15 also had similar patterns. The presence of a 5.3 kb band in the former group of ribotypes was the only difference between those two groups.

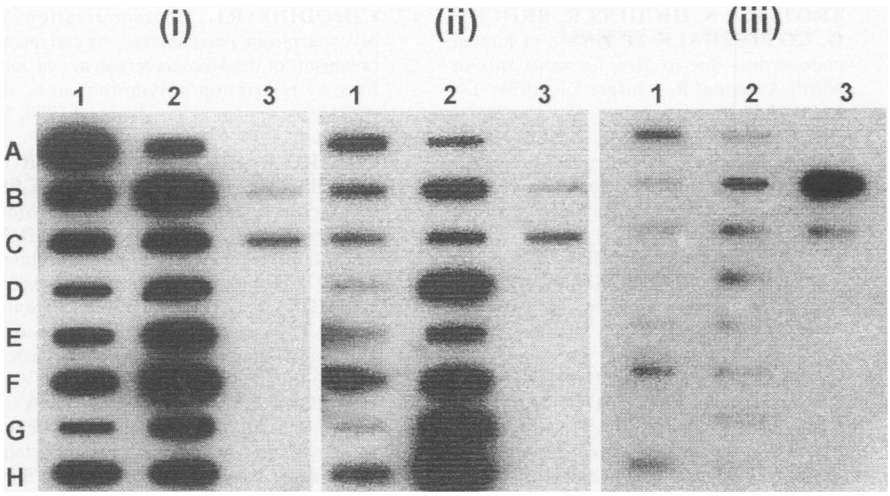

Fig. 1. Slot-blot DNA-DNA hybridization reference strains of *Streptococcus suis* capsular type 2 (lane A1), 3 (lane B1), 4 (lane C1), 5 (lane D1), 9 (lane E1), 11 (lane F1), 12 (lane G1), 14 (lane H1), 15 (lane A2), 18 (lane B2), 21 (lane C2), 22 (lane D2), 23 (lane E2), 24 (lane F2), 26 (lane G2), 19 (lane H2), *E. coli* (lane A3), *S. pneumoniae* (lane B3), *S. bovis* (lane C3).
DNA genomic probes were in panel I: *S. suis* capsular type 2; in panel II: *S. suis* capsular type 22; and in panel III: *S. pneumoniae*.

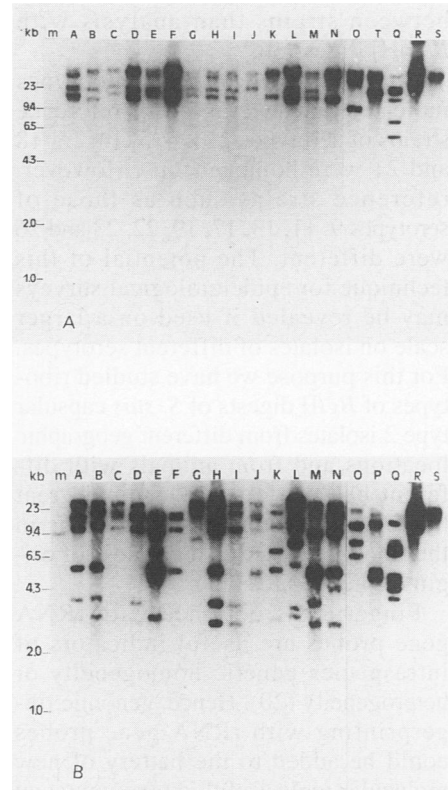

Fig. 2. Southern blot of *Bam*HI (panel A) and *Bgl*I (panel B) digested DNAs from reference strains of Streptococcus suis . Blots were probed with (^{32}P)-labelled rDNA probe. The size of lambda digest molecular markers (lane m) is indicated in kb. Reference strains of *S. suis* capsular type 2 (lane A), 3 (lane B), 4 (lane C), 5 (lane D), 9 (lane E), 11 (lane F), 12 (lane G), 14 (lane H), 15 (lane I), 18 (lane J), 21 (lane K), 22 (lane L), 23 (lane M), 24 (lane N), 26 (lane O), 19 (lane P), 25 (lane T), *E. coli* (lane Q), *S. pneumoniae* (lane R), *S. bovis* (lane S).

DISCUSSION

All *S. suis* reference strains have similar basic biochemical profiles, making differentiation of various capsular types difficult (15). However, using biochemical tests only, some isolates can be misdiagnosed (15). Since some capsular type 22 isolates are atypical and could be misidentified as *S. pneumoniae* (15), we wanted to study the genetic relationship of the latter with *S. suis* serotypes. DNA from *S. suis* serotypes 2 and 22 hybridized with the DNA of all tested serotypes, but to a lesser extent with DNA from *S. bovis*, but not with DNA from *S. pneumoniae*, or *E. coli*. This observation confirms the genetic relatedness among *S. suis* serotypes. Using *S. suis* serotypes 1, 4 and 15 reference strains as DNA probes in liquid hybridization studies, DNA-DNA relatedness values among strains of *S. suis* of serotypes 1 to 8 and 1/2 with these probes averaged between 75 to 98%; DNA-DNA relatedness values between strains of *Streptococcus viridans*, and *Streptococcus oralis* and *S. suis* with these probes averaged from 10% to 40% (10). We observed variations in the intensity of some hybridization signals using genomic probes of *S. suis* serotypes 2 and 22, with some reference strains of *S. suis*; this might be indicative of a lower degree of relatedness. Even though capsular type 22 strains are biochemically similar to *S. pneumoniae*, our hybridization studies demonstrated that capsular types 2 and 22 are genetically related; but different from *E. coli* and *S. pneumoniae*. Moreover, preliminary data on free solution DNA-DNA hybridization used to establish the genetic relationship indicated that a high degree of genetic relatedness (74% to 90%) was detected when representative strains of *S. suis* capsular types 2, 10, 11 and 12 were hybridized with reference strain of serotype 22 (Harel and MacInnes, unpublished observations).

The introduction of rRNA sequencing techniques has provided a major breakthrough in determining the evolution and phylogeny of prokaryotes (27, 28). Ribosomal cataloguing will provide the most exact way of detecting phylogenetic relationship amongst prokaryotes such as reference strains of *S. suis*.

Since rRNAs are conserved ubiquitous molecules in bacteria, electrophoretic analysis of rRNA gene restriction fragments has become a useful tool for accurate identification of bacterial species (20). One application of this technique is involved in phylogenetic analyses, and another application deals with molecular typing of bacterial pathogens during epidemiological studies (20). Several studies have also demonstrated that ribotyping can be useful to classify species and subspecies (22, 29) and to characterize virulent strains (18). Moreover, ribotyping of *Campylobacter jejuni* and *Campylobacter coli* was shown to be as discriminatory as Penner serotyping system (29). This technique has already been used to compare strains of other streptococcal species (30, 31).

The comparison of the restriction patterns showed a genetic diversity among *S. suis* isolates of different capsular types. Using DNA digested with *Bam*HI and *Bgl*II for ribotyping, we found some bands were shared between most strains, but variations among *S. suis* strains of different capsular types were observed. We identified 12 different *Bgl*II DNA patterns among 17 serotypes and nine different *Bam*HI DNA patterns. Common bands were seen with *Bgl*II in the region of 21, 13, 10.5 and 2.9 kb. Common bands were seen with *Bam*HI in the region of 21, 14, 12 kb. Thus, the choice of enzyme for ribotyping may determine the heterogeneity of patterns. Analysis based on *Bgl*II digestion allowed a better discrimination

between strains than analysis with *Bam*HI digestion.

Analysis based on *Bgl*II ribotypes indicated that several *S. suis* reference strains of serotypes 2, 3, 4, 5, 14, 15, 18 and 21 were homogeneous. However, reference strains such as those of serotypes 9, 11, 13, 17, 19, 22, 23 and 26 were different. The potential of this technique for epidemiological surveys may be revealed if used on a larger scale on isolates of different serotypes. For this purpose we have studied ribotypes of *Bgl*II digests of *S. suis* capsular type 2 isolates from different geographic locations and from animals with different health status. We found great homogeneity with some variations in the ribotype patterns, regardless of origin of the isolates (11).

Fingerprints obtained with rRNA gene probes are useful indicators of intraspecies genetic homogeneity or heterogeneity (20). Hence, genomic fingerprinting with rRNA gene probes could be added to the battery of new molecular tools useful in taxonomy and in epidemiology and diagnosis of *S. suis* infections. For example, this tool as well as restriction enzyme analysis may be used to distinguish between virulent and nonvirulent strains, as well as the identification of epidemiologically important *S. suis* strains occurring in different swine herds and which could be useful in the production of vaccines.

ACKNOWLEDGMENTS

We thank Céline Forget and Mario Cantin for excellent technical assistance, Dean Cain for his help in photography, and J. I. MacInnes (University of Guelph) for reading of the manuscript. The work was supported in part by the Ministère de l'Enseignement Supérieur et de la Science du Québec and by a grant from Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (JH 93-NC-127).

REFERENCES

1. **ARENDS JP, ZANEN HC.** Meningitis caused by *Streptococcus suis* in humans. Rev Infect Dis 1988; 10: 131–137.
2. **HIGGINS R, GOTTSCHALK M, FECTEAU G, SAUVAGEAU R, DeGUISE S, DuTREMBLAY D.** Isolation of *Streptococcus suis* from cattle. Can Vet J 1990; 31: 529.
3. **TROTTIER S, HIGGINS R, BROCHU G, GOTTSCHALK M.** A case of human endocarditis due to *Streptococcus suis* in North America. Rev Infect Dis 1991; 13: 1251–1252.
4. **PERCH B, PEDERSEN KB, HENRICHSEN J.** Serology of capsulated streptococci pathogenic for pigs: Six new serotypes of *Streptococcus suis*. J Clin Microbiol 1983; 17: 993–996.
5. **GOTTSCHALK M, HIGGINS R, JACQUES M, MITTAL KR, HENRICHSEN J.** Description of 14 new capsular types of *Streptococcus suis*. J Clin Microbiol 1989; 27: 2633–2636.
6. **GOTTSCHALK M, HIGGINS R, JACQUES M, BEAUDOIN M, HENRICHSEN J.** Characterization of six new capsular types (23 through 28) of *Streptococcus suis*. J Clin Microbiol 1991; 29: 2590–2594.
7. **HIGGINS R, GOTTSCHALK M, BEAUDOIN M, RAWLUK S.** Distribution of *Streptococcus suis* capsular types in Quebec and western Canada. Can Vet J 1992; 33: 27–30.
8. **HIGGINS R, GOTTSCHALK M, MITTAL KR, BEAUDOIN M.** *Streptococcus suis* infections in swine. A sixteen month study. Can J Vet Res 1990; 54: 170–173.
9. **HOMMEZ J, DEVRIESE LA, HENRICHSEN J, CASTRYCK F.** Identification and characterization of *Streptococcus suis*. Vet Microbiol 1986; 11: 349–355.
10. **KILLPER-BALTZ R, SCHLEIFER KH.** *Streptococcus suis* sp. nov., nom. rev. Int J Syst Bacteriol 1987; 37: 160–162.
11. **BEAUDOIN M, HAREL J, HIGGINS R, GOTTSCHALK M, FRENETTE M, MacINNES JI.** Molecular analysis of isolates of *Streptococcus suis* capsular type 2 by restriction endonuclease digested DNA separated on SDS-PAGE and by hybridization with an rDNA probe. J Gen Microbiol 1992; 138: 2639–2645.
12. **MOGOLLON JD, PIJOAN C, MURTAUGH MP, KAPLAN EL, COLLINS JE, CLEARY PP.** Identification of epidemic strains of *Streptococcus suis* by genomic fingerprinting. J Clin Microbiol 1991; 29: 782–787.
13. **MOGOLLON JD, PIJOAN C, MURTAUGH MP, KAPLAN EL, COLLINS JE, CLEARY PP.** Characterization of prototype and clinically defined strains of *Streptococcus suis* by genomic fingerprinting. J Clin Microbiol 1990; 28:2462–2466.
14. **SKOJOLD SA, QUIE PG, FRIES LA, BARNHAM M, CLEARY PP.** DNA fingerprinting of *Streptococcus zooepidemicus* (Lancefield group C) as an aid to epidemiological study. J Infect Dis 1987; 155: 1145–1150.
15. **GOTTSCHALK M, HIGGINS R, JACQUES M, BEAUDOIN M, HENRICHSEN. J** Isolation and characterization of *Streptococcus suis* capsular types 9-22. J Vet Diagn Invest 1991; 3: 60–65.
16. **NORMURA M, POST LE.** Organisation of ribosomal genes and regulation of their expression in *Escherichia coli*. In: Chambliss G, Graven G, Davis K, Cahan L, Normura M, eds. Ribosomes; Structure, Function and Genetics. University Baltimore Press, 1980: 678–691.
17. **CHIODINI RJ.** Characterization of *Mycobacterium paratuberculosis* and microorganisms of the *Mycobacterium avium* complex by restriction polymorphism of the rRNA gene region. J Clin Microbiol 1990; 28: 489–494.
18. **PICARD B, PICARD-PASQUIER N, KRISHNAMOORTHY R, GOULLET PH.** Characterization of highly virulent *Escherichia coli* strains by ribosomal DNA restriction fragment length polymorphism. FEMS Microbiol Lett 1991; 82: 183–188.
19. **GRIMONT F, GRIMONT PAD.** Ribosomal ribonucleic and gene restriction patterns as potential taxonomic tools. Ann Inst Pasteur Microbiol 1986; 137B: 165–175.
20. **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.
21. **OWEN RJ.** Chromosomal DNA fingerprinting: a new method of species and strain identification applicable to microbial pathogens. J Med Microbiol 1989; 30: 89–99.
22. **STULL TL, LILIPUMA JJ, EDLIND TD.** A broadspectrum probe for molecular epidemiology of bacteria; ribosomal RNA. J Infect Dis 1988; 157: 280–286.
23. **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.
24. **LEBLANC JD, LEE LN.** Rapid screening procedure for detection of plasmids in streptococci. J Bacteriol 1979; 140:1112–1115.
25. **HAREL J, LAPOINTE H, FALLARA A, LORTIE LA, BIGRAS-POULIN M, LARIVIÈRE S, FAIRBROTHER JM.** Detection of genes for fimbrial antigens and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with diarrhea. J Clin Microbiol 1991; 29: 745–752.
26. **SAMBROOK J, FRITSCH EF, MANIATIS T.** Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 1989.
27. **STACKEBRANDT E, GOODFELLOW M.** Introduction. In: Stackebrandt E, Goodfellow M, eds. Nucleic Acid Techniques in Bacterial Systematics. New York: John Wiley & Sons, 1991.
28. **WOESE CR.** Bacterial evolution. Microbiol Rev 1987; 51: 221–271.
29. **FAYOS A, OWEN RJ, DESAI M, HERNANDEZ J.** Ribosomal RNA restriction fragment diversity amongst Lior biotypes and Penner serotypes of *Campylobacter jejuni* and *Campylobacter coli*. FEMS Microbiol Lett 1992; 95: 87–94.
30. **CLEARY PP, KAPLAN EL, LIVDHAL C, SKOJOLD S.** DNA fingerprints of *Streptococcus pyogenes* are M type specific. J Infect Dis 1988; 158: 1317–1323.
31. **MARTIN NJ, KALPAH EL, GERBER MA, MENEGUS MA, RANDOLPH M, BELL K, CLEARY P.** Comparison of epidemic and endemic group G streptococci by restriction enzyme analysis. J Clin Microbiol 1990; 28: 1881–1886.