Deoxyribonucleic Acid Hybridizations Among Strains of *Streptococcus salivarius* and *Streptococcus bovis*

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The genetic relationships among 33 streptococci that were identified as *Streptococcus salivarius* or *Streptococcus bovis* and were isolated from humans were determined by deoxyribonucleic acid-deoxyribonucleic acid hybridization on membrane filters. The phenotypic characteristics of these bacteria were determined by their action on 20 substrates in a commercially prepared system, as well as by colony morphology and conventional tests. The *S. salivarius* strains were biochemically heterogeneous but genetically homogeneous. Although there were some phenotypic similarities between *S. bovis* and *S. salivarius*, these two species were genetically distinct. Within *S. bovis* there was genetic heterogeneity. Typical *S. bovis* strains (*S. bovis* biotype I) were genetically homologous with some, but not all, of the *S. bovis* variant strains (*S. bovis* biotype II). Other *S. bovis* biotype II strains formed a separate genetic group, the members of which were biochemically somewhat different from other *S. bovis* biotype II strains.

Streptococcus bovis has become medically important since it was discovered to be a frequent cause of endocarditis (13, 19, 20, 30, 35) and to be more common in the bowels of patients with colon diseases, including carcinomas (23, 24, 31, 36). Isolates from such human sources ferment mannitol, usually ferment inulin, and produce an extracellular glucan from sucrose (13, 34). These typical strains are called *Streptococcus bovis* biotype I to differentiate them from strains of a variant type called *Streptococcus bovis* biotype II, which do not ferment mannitol or inulin and do not produce glucan (13, 34). As is usual with such divisions, there are strains whose characteristics put them midway between the biotypes (for example, mannitol fermentation but no glucan production). In addition, some members of the common human commensal *Streptococcus salivarius* resemble *Streptococcus bovis* because they have similar biochemical traits and may react with Lancefield group D antiserum or grow on bile-esculin agar (37) or both. Like *Streptococcus bovis*, *Streptococcus salivarius* produces a polymer, usually levan (21, 32), from sucrose, and the two species can produce similar colonies on sucrose agar.

To clarify the relationships among strains identified as *Streptococcus salivarius*, *Streptococcus bovis* biotype I, and *Streptococcus bovis* biotype II, we compared the base sequence similarities of their deoxyribonucleic acids (DNAs) by using DNA-DNA hybridization.

MATERIALS AND METHODS

The strains used in this study are listed in the tables (see below). Strains whose designations begin with CDC were obtained from the Centers for Disease Control, Atlanta, Ga.; strains whose designations begin with COL were obtained from the Central Public Health Laboratories, Colindale, England; and strains whose designations begin with MG were obtained from The Massachusetts General Hospital, Boston. Other strains were obtained from clinics at the University of Connecticut Health Center, Farmington. Strain 13419 was a subculture of *Streptococcus salivarius* ATCC 13419, a strain whose nutrition and extracellular polymers have been exam-

ined (5, 21). Strain TOVE-R is a *Streptococcus salivarius* strain which produces copious extracellular glucans from sucrose (40). Strain COL005 is a subculture of *Streptococcus bovis* NCTC 8133, which was a human isolate. Some of these strains were typical of *Streptococcus salivarius* or *Streptococcus bovis*, and some were biochemically, serologically, or colonially atypical (see below). We believe that all of these streptococci are of human origin. Pure cultures were established and maintained in Todd-Hewitt broth supplemented with 1% extra glucose and CaCO₃. Cultures grown for the production of DNA or for biochemical tests were Gram stained and streaked onto blood agar plates to certify their purity.

Biochemical and cultural characterization. Each strain was tested for several biochemical reactions by using the Rapid Strep system (DMS Laboratories, Flemington, N.J.), a commercial test strip system which we and other workers have found useful for the identification of streptococci (1, 7, 15, 38; Gustafson and Coykendall, manuscript in preparation). This system tests for the production of acetoin, α -galactosidase, β -galactosidase, β -glucuronidase, pyrrolidonylarylamidase, alkaline phosphatase, and leucine arylamidase, the hydrolysis of hippurate, esculin, and arginine, and the fermentation of arabinose, glycogen, inulin, lactose, mannitol, raffinose, ribose, sorbitol, starch, and trehalose. Conventional methods were used for several strains to test for growth on bile-esculin agar (26) and for the production of urease (26) and hydrogen peroxide (42). Colony morphology was observed on casein-soy agar (Trypticase soy agar; BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% sheep blood and on mitis salivarius agar (Difco Laboratories, Detroit, Mich.), which contains 5% sucrose, which promotes production of glucans and levans. Extracellular polysaccharides were not analyzed, but we recorded whether the colonies were typical of *Streptococcus salivarius* (21) or *Streptococcus bovis* (2) or were flat or low convex, which was interpreted to represent a lack of appreciable extracellular polysaccharide production. Streptococci which were identified as *Streptococcus bovis* strains were tested for the Lancefield group D antigen with a latex bead agglutination system (Streptex; Wellcome Laboratories, Research Triangle Park, N.C.). Many *Streptococcus bovis* isolates fail to react in this system (6, 14, 37).

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TABLE 1. Biochemical and cultural characteristics and DNA hybridization of *Streptococcus salivarius* strains^a

Strain	Biochemical tests							Colony morphology ^b	Rapid strep system identity ^c	% Hybridization ^d
	Voges-Proskauer	Esculin	Trehalose	Inulin	Raffinose	Starch	Urease			
AC002	+	+	—	+	+	+	—	Sal	<i>Streptococcus salivarius</i>	113
KB005	+	+	+	—	+	—	+	Sal	<i>Streptococcus salivarius</i>	100
MG561	+	+	+	+	+	+	+	None	<i>Streptococcus salivarius</i>	103
MG568	+	+	+	—	+	+	+	Sal	<i>Streptococcus salivarius</i>	119
TOVE-R	+	+	—	w	+	—	+	Bov	<i>Streptococcus salivarius</i>	102
UC8105	+	+	+	+	+	+	+	Sal	<i>Streptococcus salivarius</i>	61
CDC009	+	+	+	—	+	+	—	Sal	NI ^f	121
CM101	+	+	—	—	+	—	+	Sal	<i>Streptococcus salivarius</i>	99
CM104	+	+	+	+	+	+	—	Sal	<i>Streptococcus salivarius</i>	113
13419	+	+	+	—	+	+	—	Sal	<i>Streptococcus salivarius</i>	114
MG4	—	+	+	+	+	+	—	Sal	<i>Streptococcus pneumoniae</i>	95
COL001	—	—	+	—	—	—	—	None	<i>Streptococcus mitis</i>	97
CDC010	—	+	—	—	—	—	—	Sal	<i>Streptococcus sanguis</i> or <i>Streptococcus mitis</i>	125
CDC013	+	+	+	+	+	+	—	Sal	<i>Streptococcus salivarius</i>	88
CDC014	+	+	+	—	+	+	+	Sal	<i>Streptococcus salivarius</i>	95
CDC015	+	+	—	—	—	—	—	None	<i>Streptococcus sanguis</i> or <i>Streptococcus mitis</i>	51

^a All *Streptococcus salivarius* strains were positive for leucine arylamidase, β -galactosidase (except strain AC002), and lactose and negative for hippurate, pyrrolidonylarylamidase, α -galactosidase, β -glucuronidase, alkaline phosphatase, arginine, ribose, arabinose, mannitol, sorbitol, and glycogen (except strain CDC009).

^b Colony morphology on Mitis-salivarius agar. Sal, Colonies typical of *Streptococcus salivarius* strains; Bov, colonies resemble colonies of typical *Streptococcus bovis* biotype I strains; Sang, colonies resemble the hard adherent colonies of *Streptococcus sanguis*; None, flat or low convex colonies apparently devoid of polysaccharide.

^c Identity as determined by the Rapid Strep system.

^d Levels of hybridization with labeled DNA from strain KB005, expressed as percentages of the homologous control value.

^e +, Positive reaction; —, negative reaction; w, weak reaction.

^f NI, Not identified.

We recorded agglutination (or the lack of it) for each strain after tests were performed according to the directions of the manufacturer.

Cell lysis and extraction of DNA. The general method used to lyse streptococci has been described previously (8). Briefly, log phase cells grown in Todd-Hewitt broth supplemented with 1% extra glucose were treated with penicillin and then harvested and lysed with lysozyme and detergents. *Streptococcus salivarius* was difficult to lyse, and the method was modified as follows. One liter of broth that had been warmed overnight in an anaerobic chamber was inoculated with 100 ml of freshly grown cells. Growth was monitored by measuring optical density. The pH was periodically adjusted to about 7.0 with NaOH. In late log phase 10 U of penicillin G per ml was added, and growth was allowed to continue with pH adjustments as described above.

When growth slowed, the cells were harvested by centrifugation and washed with deionized water. The washed cells were suspended in about 30 ml of 10 mM phosphate buffer (pH 7) and incubated at 37°C for 1 h to allow catabolism of any stored polysaccharide. The cells were then centrifuged, suspended in 20 ml of 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) supplemented with 10 mg of lysozyme (Sigma Chemical Co., St. Louis, Mo.), and incubated at 37°C for 1 h. The suspension was brought to 55°C and incubated for 1 h with 200 U of mutanolysin (Sigma). Then, 10× SSC was added to bring the salt concentration to that of 1× SSC, and 0.5 ml of sodium dodecyl sulfate was added while the cell suspension was warmed to 60°C. The detergent lysed the cells, and the thick crude product was held at 60°C for about 20 min. Finally, the material was incubated overnight at 37°C with 1 ml of 5% protease from *Streptomyces griseus* (catalog no. P-5147; Sigma). The DNA

was purified by phenol and chloroform extractions, treatment with ribonuclease, and precipitations with ethanol and isopropanol (27). *Streptococcus bovis* was much easier to lyse, and mutanolysin was not needed.

For radioactively labeled DNA, 500 ml of Todd-Hewitt broth was supplemented with 1 mCi of [methyl-³H]thymidine.

Determination of DNA base contents. Base compositions (guanine-plus-cytosine [G+C] contents) were determined by thermal denaturation in 1× SSC (28). The absorbance of the DNA sample was recorded continually while the sample was heated 0.5°C/min.

DNA hybridization. DNA was hybridized on membrane filters in 30% (vol/vol) dimethyl sulfoxide in 2× SSC as described by Legault-Démare et al. (25). We proceeded as previously described (10), with one modification. DeLey and Tjigtat (12) found that some DNA is released from filters in the first hour and thus is available to compete for the labeled DNA. Therefore, we incubated the filters (with their bound DNA) for 1 h in the dimethyl sulfoxide-2× SSC solvent and then exchanged it for fresh solvent. Then 1 µg of sonically sheared [³H]DNA was added. All experiments were done in duplicate.

Reassociation was allowed to proceed for 16 h at 48°C, after which the filters were rinsed in warm solvent and dried and the amount of bound, hybridized [³H]DNA was estimated by liquid scintillation counting. Each experiment included filters bearing DNA from the same strain that supplied the labeled DNA (the homologous control) and filters bearing *Escherichia coli* DNA (the negative control) or filters with no DNA (the blank). The level of DNA homology between strains was measured by comparing the amount of [³H]DNA bound to the unlabeled DNA of each strain with the amount of [³H]DNA bound to the DNA in the homo-

TABLE 2. Biochemical and cultural characteristics of *Streptococcus bovis* strains^a

Strain	Biochemical tests									Colony morph-ology ^c	Identity as determined by:	
	β -Glucuronidase	β -Galactosidase	Man-nitol	Tre-halose	Inulin	Starch	Glycogen	Bile-esculin	Group D anti-serum ^b		Rapid strep system	DNA hybridization
CDC003	— ^d	—	+	+	+	+	+	+	+	Bov	<i>Streptococcus bovis</i> biotype I	<i>Streptococcus bovis</i> biotype I
CDC001	—	—	+	+	+	+	+	+	—	None	<i>Streptococcus bovis</i> biotype I	<i>Streptococcus bovis</i> biotype I
CDC002	—	—	+	+	+	+	+	+	—	Sang	<i>Streptococcus bovis</i> biotype I	<i>Streptococcus bovis</i> biotype I
MGEath	—	+	+	+	—	+	+	—	—	Bov	<i>Streptococcus bovis</i> biotype I	<i>Streptococcus bovis</i> biotype I
COL003	—	—	—	—	—	—	—	+	+	None	<i>Streptococcus bovis</i> biotype II/1 or <i>Streptococcus salivarius</i>	<i>Streptococcus bovis</i> biotype II/1
CDC011	—	—	—	—	—	—	—	+	—	None	<i>Streptococcus bovis</i> biotype II/1 or <i>Streptococcus salivarius</i>	<i>Streptococcus bovis</i> biotype II/1
CDC012	—	—	—	—	—	+	+	+	+	None	<i>Streptococcus bovis</i> biotype II/1 or <i>Streptococcus salivarius</i>	<i>Streptococcus bovis</i> biotype II/1
COL005	—	w	—	—	—	+	—	+	—	Bov	NI ^e	<i>Streptococcus bovis</i> biotype II/1
MGFras	—	+	—	—	—	+	—	+	—	None	<i>Streptococcus bovis</i> biotype II/1 or <i>Streptococcus salivarius</i>	<i>Streptococcus bovis</i> biotype II/1
CDC018	—	—	—	—	—	+	—	—	—	Sal	<i>Streptococcus bovis</i> biotype II/1	<i>Streptococcus bovis</i> biotype II/1
CDC008	+	+	—	+	—	—	—	+	+	Sal	<i>Streptococcus bovis</i> biotype II/2	<i>Streptococcus bovis</i> biotype II/2
COL002	+	+	—	+	—	+	—	+	—	None	<i>Streptococcus bovis</i> biotype II/2	<i>Streptococcus bovis</i> biotype II/2
MG6	—	w	—	+	—	—	—	+	+	None	<i>Streptococcus bovis</i> biotype II/2 or <i>Streptococcus salivarius</i>	<i>Streptococcus bovis</i> biotype II/2
MGSoph	+	+	—	+	—	—	—	+	+	Sal	<i>Streptococcus bovis</i> biotype II/2	<i>Streptococcus bovis</i> biotype II/2
MGLars	—	—	—	+	—	—	—	+	+	None	<i>Streptococcus bovis</i> biotype II/2 or <i>Streptococcus salivarius</i>	<i>Streptococcus bovis</i> biotype II/2
MG20	—	—	—	+	—	—	—	+	w	None	<i>Streptococcus bovis</i> biotype II/2 or <i>Streptococcus salivarius</i>	<i>Streptococcus bovis</i> biotype II/2

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TABLE 2—Continued

Strain	Biochemical tests									Colony morphology ^c	Identity as determined by:	
	β -Glucuronidase	β -Galactosidase	Mannitol	Trehalose	Inulin	Starch	Glycogen	Bile-esculin	Group D anti-serum ^b		Rapid strep system	DNA hybridization
MG565	—	—	—	+	—	—	—	+	—	None	<i>Streptococcus bovis</i> biotype II/2 or <i>Streptococcus salivarius</i>	<i>Streptococcus bovis</i> biotype II/2
CDC007	+	+	—	+	—	—	—	+	—	None	<i>Streptococcus bovis</i> biotype II/2 or <i>Streptococcus salivarius</i>	Uncertain

^a All *Streptococcus bovis* strains were positive for acetoin, esculin, α -galactosidase, leucine arylamidase, lactose, and raffinose and negative for hippurate, pyrrolidonylarylamidase, alkaline phosphatase, arginine, ribose, arabinose (except strain COL005), and sorbitol.

^b Reaction with Lancefield group D antiserum, as determined in a latex bead agglutination system.

^c Bov, Colonies resemble colonies of typical *Streptococcus bovis* biotype I strains; Sang, colonies resemble the hard adherent colonies of *Streptococcus sanguis*; Sal, colonies typical of *Streptococcus salivarius* strains; None, flat or low convex colonies apparently devoid of polysaccharide.

^d +, Positive reaction; —, negative reaction; w, weak reaction.

^e NI, Not identified.

gous control; values were expressed as percentages, the homologous control value being 100%.

In the first experiments, type HAWP filters (25 mm; Millipore Corp., Bedford, Mass.) were used, but in later experiments we used type HAHY filters. We found that these filters bound about twice as much single-stranded DNA as the type HAWP filters and released proportionally less DNA during incubation.

RESULTS

Characteristics of *Streptococcus salivarius*. Table 1 lists all of the strains that were identified as *Streptococcus salivarius* by biochemical tests or DNA homology or both, along with one strain of uncertain identity. All of these streptococci were positive for leucine arylamidase, β -galactosidase (except strain AC002), and lactose and negative for hippurate, pyrrolidonylarylamidase, β -glucuronidase, alkaline phosphatase, arginine, ribose, arabinose, mannitol, sorbitol, and glycogen (except strain CDC009). The reactions that were variable, colony morphology, and the extents of DNA hybridization as percentages of the homologous control are shown in Table 1. The Rapid Strep system gave an incorrect identification if a strain failed to produce acetoin (Voges-Proskauer test) and did not accept strain CDC009 because of its acidification of glycogen. Strain CDC015 fermented few sugars and was not identified; the identity of this strain was uncertain by DNA also. Of the 14 strains that were genetically related, only strain COL001 had neither the colony morphology nor the biochemical profile of *Streptococcus salivarius*. We believe that strain UC8105 is a *Streptococcus salivarius* strain and suspect that its DNA preparation was of poor quality.

Table 1 does not show the levels of homology between strain KB005 DNA and DNAs from six *Streptococcus bovis* strains. All of these values were very low (between 25 and 38%).

***Streptococcus bovis* biotypes.** The strains identified as *Streptococcus bovis* and their phenotypic characters are shown in Table 2, and the DNA hybridization data for these strains are shown in Table 3. All *Streptococcus bovis* strains were positive for acetoin, esculin, and α -galactosidase and

negative for hippurate, pyrrolidonylarylamidase, alkaline phosphatase, arginine, ribose, arabinose (except strain COL005), and sorbitol. Four strains were typical of *Streptococcus bovis* biotype I in their ability to ferment mannitol. One strain did not grow on bile-esculin agar, and two had nontypical colony morphologies.

Streptococcus bovis biotype II strains did not ferment mannitol or inulin, and most did not ferment starch or glycogen. The Rapid Strep system recognized two subtypes of *Streptococcus bovis* biotype II. Six of our strains were identified as *Streptococcus bovis* biotype II/1, and eight were called *Streptococcus bovis* biotype II/2. There were very few differences between *Streptococcus bovis* biotype II/1 and *Streptococcus bovis* biotype II/2. One-half of the biotype II/2 strains but none of the biotype II/1 strains produced β -glucuronidase. Fermentation of starch and glycogen was more common among biotype II/1 strains. The most consistent biochemical difference between the two biotypes involved fermentation of trehalose. All *Streptococcus bovis* biotype II/2 strains acidified this sugar, but *Streptococcus bovis* biotype II/1 strains did not.

In many cases the identification of *Streptococcus bovis* biotype II strains was not explicit when the Rapid Strep system was used. In each case the bacterium had a biochemical profile similar to that of *Streptococcus salivarius*. However, the "percent identification," a term used by the system to express the level of confidence in an identification, always favored *Streptococcus bovis* over *Streptococcus salivarius* in these equivocal cases. Interestingly, the Rapid Strep system never confused *Streptococcus bovis* biotype II/1 with *Streptococcus bovis* biotype II/2. One strain, strain COL005, was not identified. Presumably this was because it fermented arabinose; it was otherwise typical of *Streptococcus bovis* biotype II/1 strains. Fermentation of arabinose is common in *Streptococcus bovis* strains isolated from animals (see below).

The variety of colony forms produced by both *Streptococcus bovis* and *Streptococcus salivarius* on sucrose agar indicated that colonial morphology alone is not reliable for identifying these streptococci. None of the strains was β -hemolytic. As noted by other workers (6, 17, 37), many *Streptococcus bovis* strains do not react with group D

TABLE 3. DNA-DNA Hybridization among strains of *Streptococcus bovis*^a

Source of unlabeled DNA	% Hybridization with labeled DNA from:			
	<i>Streptococcus bovis</i> biotype I strain CDC003		<i>Streptococcus bovis</i> biotype II/1 strain COL003	<i>Streptococcus bovis</i> biotype II/2 strain CDC008
	Expt 1	Expt 2		
<i>Streptococcus bovis</i> biotype I				
CDC003	100	100	38	90
CDC001	102			
CDC002	100			
MGEthe	123			
<i>Streptococcus bovis</i> biotype II/1				
COL003		56	100	
CDC011	61	32	116	73
CDC012			76	
COL005		56	84	
MGFras	71		117	75
CDC018	55		103	55
<i>Streptococcus bovis</i> biotype II/2				
CDC008	85	63	40	100
COL002	85	127		92
MG6		113		
MGSoph		87		
MGLars		106		
MG20		99		
MG565		97		
CDC007		40		
<i>E. coli</i>	7	9	8	9
Blank	8	8	7	

^a Data are expressed as percentages of the homologous control value.

antibody in the commercial agglutination system that we used.

Hybridization among *Streptococcus bovis* biotypes. The four strains of *Streptococcus bovis* biotype I were genetically homologous with each other (Table 3). The same experiment indicated that some strains of *Streptococcus bovis* biotype II were homologous with *Streptococcus bovis* biotype I, since their DNAs hybridized extensively (85%) with labeled DNA of *Streptococcus bovis* biotype I strain CDC003. Additional experiments showed that all strains of *Streptococcus bovis* biotype II/2 (except strain CDC007) were homologous with each other and with *Streptococcus bovis* biotype I strain CDC003, but not with strains of *Streptococcus bovis* biotype II/1 and that *Streptococcus bovis* biotype II/1 strains formed a separate group whose DNAs all hybridized well with labeled DNA from *Streptococcus bovis* biotype II/1 strain COL003, whereas DNAs from *Streptococcus bovis* biotype I strain CDC003 and *Streptococcus bovis* biotype II/2 strain CDC008 did not. Two experiments (data not shown) showed that *Streptococcus bovis* biotype II/2 strain CDC007 was not related to *Streptococcus salivarius* or *Streptococcus bovis* biotype II/1. The failure of the DNA of this strain to hybridize with other *Streptococcus bovis* biotype II/2 DNAs may have been purely technical. DNA from *Streptococcus bovis* never hybridized appreciably with *Streptococcus*

salivarius DNA; the highest value was 36%, and all other values were below 30%.

Overall, the identifications of the senders agreed with identifications based on DNA data or the Rapid Strep system or both, but hybridization clarified the identities of some atypical isolates. Strain CDC009 was like *Streptococcus bovis* biotype II with respect to glycogen acidification but was actually a *Streptococcus salivarius* strain, and strain CDC018, which resembled *Streptococcus salivarius* because of its colonies on sucrose agar and its failure to grow on bile-esculin agar, proved to be a *Streptococcus bovis* biotype II/1 strain. The production of glucan by strain COL005 (= NCTC 8133) is probably the reason why this strain was originally classified as a *Streptococcus bovis* biotype I strain.

DNA base contents. There were no differences in the DNA base contents of the *Streptococcus bovis* genotypes or *Streptococcus salivarius* strains. The values obtained for *Streptococcus salivarius* strains were 43.4 ± 1.31 mol% G+C (mean $\pm$ standard deviation; range, 41.7 to 45.3 mol%); the values obtained for *Streptococcus bovis* biotype I were 42.8 ± 1.18 mol% G+C (range, 41.8 to 44.7 mol%); the values obtained for *Streptococcus bovis* biotype II/1 were 42.2 ± 1.08 mol% G+C (range, 41.4 to 44.4 mol%); and the values obtained for *Streptococcus bovis* biotype II/2 were 42.7 ± 1.04 mol% G+C (range, 40.5 to 43.6 mol%).

DISCUSSION

Our results show that *Streptococcus salivarius* strains are genetically quite homologous despite some phenotypic heterogeneity and that this species is not related to *Streptococcus bovis*. *Streptococcus bovis* biotypes I and II/2 are genetically the same and represent a single species. *Streptococcus bovis* biotype II/1 strains are distinctly different. The levels of hybridization of the DNAs of these strains with biotype I and II/2 DNAs were at most 70 to 75% and usually were lower. When *Streptococcus bovis* biotype II/1 strain COL003 DNA was the labeled DNA, the level of hybridization with *Streptococcus bovis* biotype I strain CDC003 DNA or *Streptococcus bovis* biotype II/2 strain CDC008 DNA was about 40%. Thus, there are some DNA base sequences in biotype II/1 strains that are similar to sequences in biotype I and II/2 strains. At this level of genetic similarity we would consider biotype II/1 strains members of a subspecies of *Streptococcus bovis*.

All of our *Streptococcus bovis* strains were of human origin, and our results should not be extrapolated to the *Streptococcus bovis* strains isolated from animals. Animal strains are biochemically somewhat different; many ferment arabinose but not mannitol, and glucan production correlates more with arabinose fermentation than with mannitol fermentation (11, 29). It was these streptococci, not the mannitol fermenters, that Orla-Jensen called *Streptococcus bovis* (33, 39; reviewed by Kiel and Skadhauge [22], who cited Friedberg [17]). Furthermore, the animal isolates themselves are genetically heterogeneous (18).

Our results demonstrate the following phenomena that we and other workers have observed in streptococci: there can be genetic heterogeneity among phenotypically similar streptococci, and there can be genetic homology in streptococci that are phenotypically diverse. There is great genetic diversity among the phenotypically similar members of the mutans group (4, 9). Conversely, Welborn et al. (41) found genetic homogeneity among six strains of the *Streptococcus constellatus-Streptococcus intermedius* group (or "*Streptococcus milleri*" [3]) that were heterogeneous with respect to hemolysis, Lancefield group, and action on lactose, esculin,

and starch. In the case of *Streptococcus bovis*, we found genetic homology between two distinct biotypes (biotypes I and II/2) but a lack of strong relationship between two biotypes that have only a few biochemical differences (biotypes II/1 and II/2). Our results also show that within *Streptococcus salivarius* there are strains that are hard to recognize as *Streptococcus salivarius*, yet are genetically homologous with typical strains. Moreover, *Streptococcus salivarius* DNA is indistinguishable from *Streptococcus thermophilus* DNA (16). This phenomenon illustrates how little of the bacterial chromosome is required to produce the characteristics that we observe.

Because of the relationship between *Streptococcus bovis* and colon diseases (23, 24, 31, 36), it is important to differentiate *Streptococcus bovis* from *Streptococcus salivarius* in clinical isolates. Typical strains and isolates of *Streptococcus salivarius* and *Streptococcus bovis* are not difficult to identify. However, there are isolates of *Streptococcus salivarius* that grow on bile-esculin medium, and some produce group D reactions and thus mimic *Streptococcus bovis*. Conversely, some *Streptococcus bovis* isolates are bile-esculin medium negative, and many do not give a group D reaction with commonly used agglutination systems (6, 15, 37). These problems were reviewed by Ruoff et al. (37), who found that the group D reaction of *Streptococcus salivarius* was caused by a cross-reacting antigen and that the true group D antigen of *Streptococcus bovis* could be demonstrated in hot acid extracts. In some cases the antigen required concentration by alcohol precipitation. These authors further emphasized the importance of an adequate number of tests to insure proper identification of these two species.

Although we do not recommend a commercial rapid identification system for strictly describing species of streptococci, the one used in this study was useful for identification, and it provided biochemical profiles which helped to delineate the two genetic groups of *Streptococcus bovis*.

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