

Nucleic Acid Hybridization Studies and Deoxyribonucleic Acid Base Compositions of Anaerobic, Gram-Positive Cocci

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Deoxyribonucleic acid-deoxyribonucleic acid reassociation and deoxyribonucleic acid-ribosomal ribonucleic acid cistron similarity studies showed that the anaerobic, gram-positive cocci comprise a rather heterogeneous group of bacteria. The deoxyribonucleic acid-ribosomal ribonucleic acid hybridization studies distinguished seven groups. Groups 1 and 2 consisted of *Peptostreptococcus magnus* and *Peptostreptococcus prevotii*, respectively. *Peptostreptococcus asaccharolyticus* ATCC 14963^T (T = type strain) and *Peptostreptococcus indolicus* ATCC 29427^T formed a third group, and *Peptostreptococcus asaccharolyticus* DSM 20364 together with Hare group VIII strain NCTC 9820 formed group 4. *Peptostreptococcus anaerobius* DSM 20357 was more closely related to *Eubacterium tenue* ATCC 25553^T and *Clostridium lituseburense* ATCC 25759^T than to any of the other species studied. *Peptostreptococcus micros* strains DSM 20468^T and DSM 20367 together with strains belonging to Hare group IX formed group 6, and group 7 consisted of *Peptostreptococcus parvulus* DSM 20469^T. Strains of different Hare groups were all assigned to one of the seven groups or to the genus *Staphylococcus* (Hare group VIIb strain NCTC 9819) or the genus *Streptococcus* (Hare group VIa strain NCTC 9806). The anaerobic cocci also have a diversity of murein structures. This is the first report showing that strains belonging to the same species have different murein types.

Within the last three decades it has been recognized that anaerobic, gram-positive cocci participate in a large number of infectious diseases and are frequently isolated from mixed infections (18, 20, 41, 43). Although many schemes have been proposed (5, 14, 19, 30, 31), the current classification of anaerobic cocci is unsatisfactory, and little is known about the actual relatedness of these organisms despite many studies (3, 6, 10-12, 24, 25). In the early 1950s, Hare and colleagues (18, 39) proposed a classification scheme dividing the strains into nine groups, the so-called Hare groups, on the basis of biochemical reactions. Strains belonging to group I were believed to be potential pathogens, whereas the strains of the other groups seemed to have less pathological importance.

Most of the anaerobic, gram-positive cocci can be assigned to *Peptococcus* or *Peptostreptococcus*, which, according to Rogosa (31), are distinguished mainly by their different morphologies. Very recently, however, Ezaki et al. (12) proposed, mainly on the basis of the guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs), the transfer of *Peptococcus indolicus*, *Peptococcus asaccharolyticus*, *Peptococcus prevotii*, and *Peptococcus magnus* to the genus *Peptostreptococcus*.

The aim of this study was to determine the genetic relatedness of various anaerobic, gram-positive cocci with known murein types by using nucleic acid hybridization techniques.

MATERIALS AND METHODS

Strains and growth conditions. The strains used in this study are listed in Table 1. *Escherichia coli* K-12 was grown aerobically in yeast extract-glucose-peptone broth. All other strains were cultivated anaerobically in a medium containing (per liter of distilled water) 10 g of tryptone (Oxoid Ltd.), 10 g of yeast extract (Oxoid), 2.5 g of glucose, 2.5 g of glycine, 2.5 g of glutamic acid, 3 g of sodium acetate, 5 g of sodium

chloride, 0.5 g of L-cysteinium hydrochloride, 5 mg of hemin, 0.5 mg of menadoine (vitamin K₃), 1 mg of vitamin K₁, and 1 ml of Tween 80 (Serva); the pH was adjusted to 7.2 to 7.4. The incubation temperature was 37°C.

DNA and rRNA preparation. DNA was isolated by the method of Huss et al. (22). Ribonucleic acid was labeled in vivo by adding 1 mCi of [5,6-³H]uracil (43.4 Ci/mmol; New England Nuclear Corp.) to 500 ml of growth medium without yeast extract. After the uptake of radioactivity reached a maximum, the cells were harvested, and labeled ribosomal ribonucleic acid (rRNA) was isolated by the method of Stackebrandt et al. (36).

G+C content of DNA and hybridization experiments. The melting temperature of each DNA was determined with a Gilford model 2600 spectrophotometer, and DNA base composition was calculated by the method of De Ley (7). The renaturation rate method of De Ley et al. (8) or the membrane filter technique was employed for the DNA-DNA hybridization experiments, using the conditions described by Huss et al. (22). DNA-rRNA hybridization studies were carried out by the method of Kilpper-Bälz and Schleifer (26).

Murein types. Murein types were determined by the method of Schleifer and Kandler (33).

RESULTS

G+C contents and DNA-DNA hybridization. The G+C contents of all of the strains investigated are listed in Table 1. A recent study on the cell wall structures of anaerobic gram-positive cocci (40) indicated that these organisms constitute a rather heterogeneous group with respect to murein types. We selected strains from groups with different murein types (Table 1) to elucidate the genetic relationships within and among such groups. All of the murein types were examined. The DNA-DNA hybridization data in Table 2 were determined by the membrane filter technique under optimal conditions at 25°C below the melting temperature (22). With this method, a degree of binding value (*D* value) down to about 10% is still meaningful for relatedness at the genus

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TABLE 1. List of organisms studied, their murein structures, and the G+C contents of their DNAs

Organism	Strain ^a	Murein structure				Reference	G+C content (mol%) ^b
		Peptide subunits		Interpeptide bridge			
		Position 1	Position 3				
<i>Staphylococcus saccharolyticus</i>	Evans 75-166	Ala	Lys	Gly ₂ -Ser-Gly ₂	25	33.0	
Hare group VIIb	NCTC 9819	Ala	Lys	Gly ₂ -Ser-Gly ₂	40	32.6	
<i>Staphylococcus simulans</i>	ATCC 27848 ^T	Ala	Lys	Gly _{4,9} -Ser _{0,2}	27	34.0	
Hare group I	NCTC 9801	Ala	Lys(Orn)	Lys-D-Glu	40	28.3	
Hare group III	NCTC 9814	Ala	Lys(Orn)	Gly ₅	38	29.2	
<i>Streptococcus intermedius</i>	ATCC 27335 ^T	Ala	Lys	Ala ₂	40	36.5	
Hare group VIa	NCTC 9806	Ala	Lys	Ala ₂	40	39.7	
<i>Peptostreptococcus anaerobius</i>	DSM 20357	Ala	Lys	D-Asp	40	34.2	
<i>Clostridium lituseburense</i>	ATCC 25759 ^T	Ala	Lys	D-Asp	36	26.7	
<i>Peptostreptococcus parvulus</i>	DSM 20469 ^T	Ala	Lys	D-Asp	40	44.0	
<i>Peptococcus niger</i>	DSM 20475 ^T	Ala	Lys	D-Asp	40	49.7	
<i>Peptostreptococcus indolicus</i>	ATCC 29427 ^T	Ala	Orn	D-Glu	40	30.4	
<i>Peptostreptococcus asaccharolyticus</i>	ATCC 14963 ^T	Ala	Orn	D-Glu	34	29.8	
	DSM 20364	Ala	Orn	D-Glu	23	35.6	
<i>Peptostreptococcus magnus</i>	ATCC 14955	Gly	Lys	Gly	34	32.1	
	ATCC 15794 ^T	Gly	Lys	Gly	40	32.4	
	DSM 20362	Gly	Lys	Gly	40	32.1	
	DSM 20369	Gly	Lys	Gly	40	32.3	
	ATCC 29328	Gly	Lys	Gly	40	31.6	
Hare group IV	NCTC 9804	Gly	Lys	Gly	40	32.5	
<i>Peptostreptococcus prevotii</i>	ATCC 9321 ^T	Gly	Lys	D-Glu	34	33.0	
	DSM 20358	Gly	Lys	D-Glu	40	34.0	
Hare group IX	NCTC 9811	Gly	Lys	D-Asp	40	33.0	
	NCTC 9821	Gly	Lys	D-Asp	40	30.0	
Hare group VIII	NCTC 9810	Gly	Lys	Gly-D-Asp	40	30.8	
	NCTC 9820	Gly	Lys	Gly-D-Asp	40	34.6	
<i>Peptostreptococcus micros</i> (labeled <i>Peptostreptococcus magnus</i>)	DSM 20367 ^c	Gly	Orn	D-Asp	40	27.4	
<i>Peptostreptococcus micros</i>	DSM 20468 ^T	Gly	Orn	D-Asp	40	27.5	
<i>Streptococcus sanguis</i>	DSM 20066	Ala	Lys	None	33	42.6	
<i>Eubacterium tenue</i>	ATCC 25553 ^T	Ala	m-A ₂ pm	None	37	26.1	
<i>Clostridium butyricum</i>	DSM 552 ^T	Ala	m-A ₂ pm ^d	None	37	26.8	

^a ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung für Mikroorganismen, München, Federal Republic of Germany; Evans, C. A. Evans, University of Washington, Seattle; NCTC, National Collection of Type Cultures, London, England.

^b *Escherichia coli* K-12 (G+C content, 51.7 mol% [7]) was used as the reference.

^c This strain is not listed in the latest catalog of the Deutsche Sammlung für Mikroorganismen, but it is still available (N. Weiss, personal communication).

^d m-A₂pm, meso-Diaminopimelic acid.

level, whereas the renaturation rate method (Tables 3 through 6) has no relevance below a *D* value of 25% (22). Therefore, *D* values in this range are reported as <25%.

As shown in Table 2, Hare group VIIb strain NCTC 9819 is a *Staphylococcus saccharolyticus* strain (24, 25), whereas Hare group III strain NCTC 9814 showed no detectable relatedness to *Staphylococcus saccharolyticus* or *Staphylococcus simulans* despite a pentaglycine interpeptide bridge in its murein, which is characteristic of many staphylococci (32). *Peptostreptococcus prevotii* ATCC 9321^T (T = type strain) and *Peptostreptococcus prevotii* DSM 20358 are not identical but are closely related (Table 2). The DNAs of both

of these strains showed no significant hybridization with the DNA of *Peptostreptococcus asaccharolyticus* ATCC 14963^T or the DNA of *Staphylococcus saccharolyticus* (Table 2).

The comparative physiological and biochemical investigations of Barnes et al. (1) with representatives of Hare group VIa and *Streptococcus intermedius* revealed a close relationship, which we confirmed by finding a *D* value of 53% between the DNAs of strain NCTC 9806 and *Streptococcus intermedius* ATCC 27335^T.

Various strains of *Peptostreptococcus magnus*, as well as Hare group IV, have the same murein type (Table 1). Hybridization values (*D* values) of 55 to 74% showed that

TABLE 2. DNA-DNA hybridization studies with *Peptostreptococcus prevotii*, *Peptostreptococcus asaccharolyticus*, staphylococci, and representatives of Hare groups VIIb and III^a

Source of [³ H]DNA	D value (%) with filter-bound DNA from:						
	<i>Peptostrep- toccoccus prevotii</i> ATCC 9321 ^T	<i>Peptostrep- toccoccus prevotii</i> DSM 20358	<i>Peptostrep- toccoccus asaccharolyticus</i> ATCC 14963	Hare group VIIb strain NCTC 9819	<i>Staphylococcus saccharolyticus</i> Evans 75-166	<i>Staphylococcus simulans</i> ATCC 27848 ^T	Hare group III strain NCTC 9814
<i>Peptostreptococcus prevotii</i> ATCC 9321 ^T	100	49	9	7	4	ND ^b	ND
<i>Peptostreptococcus prevotii</i> DSM 20358	50	100	8	6	5	ND	ND
<i>Peptostreptococcus asaccharolyticus</i> ATCC 14963 ^T	9	8	100	6	6	ND	ND
Hare group VIIb strain NCTC 9819	6	4	6	100	92	10	2
<i>Staphylococcus saccharolyticus</i> Evans 75-166	6	5	5	95	100	21	ND
<i>Staphylococcus saccharolyticus</i> ATCC 14953 ^T	5	6	7	ND	100	24	ND
<i>Staphylococcus simulans</i> ATCC 27848 ^T	ND	ND	ND	13	12	100	3
Hare group III strain NCTC 9814	ND	ND	ND	2	4	3	100

^a The levels of hybridization on membrane filters were determined under optimal conditions (25°C below the melting temperature [23]).

^b ND, Not determined.

these organisms form a rather homogenous group (Table 3).

Four different types of peptidoglycan have been reported for strains labeled *Peptostreptococcus prevotii* and members of Hare groups I, III, and VIII (40). Nevertheless, two strains (strains NCTC 9801 [Hare group I] and NCTC 9814 [Hare group III]) showed a high D value (77%). All of the other homology values within this group were near the limit of sensitivity of the hybridization method used (Table 4). The significance of these values is demonstrated by the results of the DNA-rRNA hybridization studies (see below).

Peptostreptococcus asaccharolyticus ATCC 14963^T is related to *Peptostreptococcus indolicus* ATCC 29427^T (D value, 38%) whereas no significant DNA homology has been observed with strain DSM 20364 (also labeled *Peptostrep-*

toccoccus asaccharolyticus) (Table 5) or four clinical isolates that are highly related to strain DSM 20364 (22; data not shown). Hare group VIII strain NCTC 9820, which has a different type of murein (Table 1), also belongs to this group (Table 5).

Strain DSM 20367 (labeled *Peptostreptococcus magnus*) has been identified as *Peptostreptococcus micros* (40). This was confirmed by the D value (81%) between the DNAs of these organisms (Table 6). Strains belonging to Hare group IX also are related to *Peptostreptococcus micros* (D values, 38 to 57%); the significance of the 28% DNA homology between *Peptostreptococcus parvulus* and *Peptostreptococcus anaerobius* was tested by DNA-rRNA hybridization (see below).

None of the other DNA-DNA hybridization experiments performed between members of the different groups listed in Table 1, including *Peptococcus niger* DSM 20475^T, indicated any significant relatedness (D values, <25% [data not shown]).

DNA-rRNA hybridization. Since the cistrons coding for rRNAs have changed considerably less during evolution than the rest of the genomes (15, 42), DNA-rRNA hybridization studies are more useful for elucidating genetic relationships at the higher levels (i.e., genus or family level) than DNA-DNA comparisons (29). In contrast to DNA-DNA reassociation experiments, the D value between DNA and rRNA is not necessarily a relevant criterion for determining

TABLE 3. DNA-DNA hybridization studies with the *Peptostreptococcus magnus* group, using the renaturation rate method

Strain	D value (%) with:					
	<i>Peptostreptococcus magnus</i> strains				Hare group IV strains	
	ATCC 15794 ^T	DSM 20362	DSM 20369	ATCC 29328	NCTC 9804	NCTC 9815
<i>Peptostreptococcus magnus</i> ATCC 14955	65	62	67	56	63	65
<i>Peptostreptococcus magnus</i> ATCC 15794 ^T		63	69	59	71	74
<i>Peptostreptococcus magnus</i> DSM 20369			70	63	65	65
<i>Peptostreptococcus magnus</i> DSM 20362				55	70	73
<i>Peptostreptococcus magnus</i> ATCC 29328					61	55
Hare group IV strain NCTC 9804						74

TABLE 4. DNA-DNA hybridization studies with *Peptostreptococcus prevotii* DSM 20358 and Hare groups I, III, and VIII (strain NCTC 9810), using the renaturation rate method

Strain	D value (%) with:		
	Hare group I strain NCTC 9801	Hare group III strain NCTC 9814	Hare group VIII strain NCTC 9810
<i>Peptostreptococcus prevotii</i> DSM 20358	28	26	<25
Hare group I strain NCTC 9801		77	25
Hare group III strain NCTC 9814			26

TABLE 5. DNA-DNA hybridization studies with *Peptostreptococcus indolicus*, *Peptostreptococcus asaccharolyticus*, and Hare group VIII (strain NCTC 9820), using the renaturation rate method

Strain	D value (%) with:		
	ATCC 14963 ^T	DSM 20364	Hare group VIII strain NCTC 9820
<i>Peptostreptococcus indolicus</i> ATCC 29427 ^T	38	<25	<25
<i>Peptostreptococcus asaccharolyticus</i> ATCC 14963 ^T		<25	<25
<i>Peptostreptococcus asaccharolyticus</i> DSM 20364			54

relationships among bacteria, since rRNA cistrons can be found in different copy numbers (29, 44, 45). A better criterion is the thermal stability of DNA-rRNA hybrids (9). The difference in melting points between homologous and heterologous hybrids [$\Delta T_{m(e)}$] was used to construct an average linkage dendrogram, which shows the relationships within the anaerobic, gram-positive cocci with respect to the thermal stability of DNA-rRNA hybrids (Fig. 1). The thermal stability values are listed in Table 7.

DISCUSSION

Figure 1 shows that the anaerobic, gram-positive cocci examined in this study can be divided into seven groups. The first group comprises strains of *Peptostreptococcus magnus*, and the second group consists of *Peptostreptococcus prevotii* and three strains belonging to different Hare groups. The third group is made up of *Peptostreptococcus asaccharolyticus* ATCC 14963^T and *Peptostreptococcus indolicus*, which have a DNA homology value (*D* value) of 38%. *Peptostreptococcus asaccharolyticus* DSM 20364 and Hare group VIII strain NCTC 9820 form a separate group. The latter two groups are characterized by having exclusively class I fructose-1,6-biphosphate aldolase, in contrast to all of the other peptostreptococci examined, which possess class II fructose-1,6-biphosphate aldolase (13). Class I fructose-1,6-biphosphate aldolase of the *Peptostreptococcus asaccharolyticus* type has otherwise been found only in staphylococci (13, 17). Our results are not in complete agreement with those of Ezaki (10) and Ezaki et al. (12); we found a DNA homology value (*D* value) of 38% (40% when the membrane filter method was used) between *Peptostreptococcus asaccharolyticus* ATCC 14963^T and *Peptostreptococcus indolicus* ATCC 29427^T, in contrast to *D* values of 3 to 8% (10) and 15% (12). These two strains also differed by about 3 to 4 mol% in their G+C contents compared with our results. The results of the cell wall analysis of our strains agreed with the

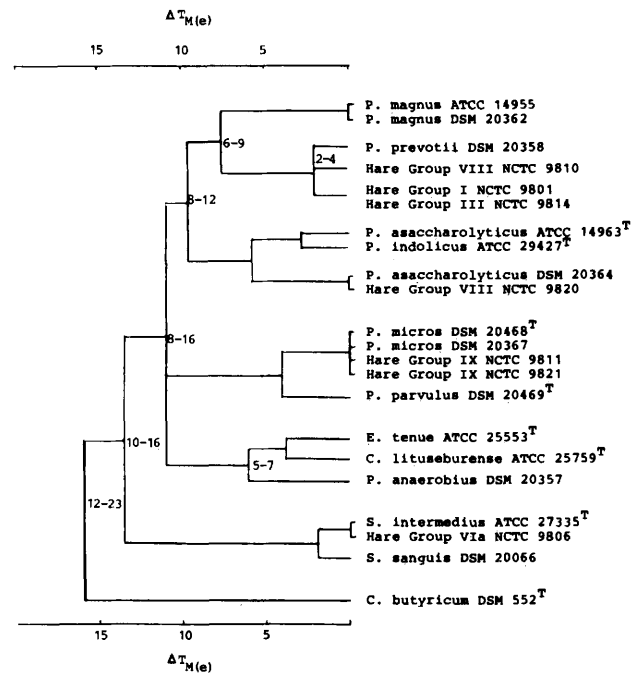


FIG. 1. Dendrogram based on DNA-rRNA hybridization studies of anaerobic, gram-positive bacteria. The $\Delta T_{m(e)}$ values of DNA-rRNA hybrids (see Table 7) were used to construct an average linkage dendrogram. The numbers at the branches reflect the spreading of $\Delta T_{m(e)}$ values between groups. The brackets indicate DNA homology values (*D* values) of more than 30%. *Streptococcus intermedius* and *Peptostreptococcus micros* DSM 20468^T were included on the basis of their high DNA-DNA homology values with Hare group IVa strain NCTC 9806 (53%) and *Peptostreptococcus micros* DSM 20367 (81%), respectively.

murein types described by Schleifer and Nimmermann (34) and Weiss (40). This indicates that we did not use wrong strains. In addition, Ezaki et al. (12) found a distinct relationship between *Peptostreptococcus asaccharolyticus* ATCC 14963^T and *Peptostreptococcus prevotii* ATCC 9321 (*D* value, 46%). Our data and those of Kilpper (R. Kilpper, Ph.D. thesis, Technical University of Munich, Munich, Federal Republic of Germany, 1980) do not show any DNA homology (*D* value, < 10%) and very low DNA-rRNA similarity ($\Delta T_{m(e)}$, 13°C) between these species.

Peptostreptococcus anaerobius DSM 20357 is only remotely related to the other anaerobic, gram-positive cocci. On the other hand, it is closely related to *Eubacterium tenue* and *Clostridium lituseburensis*. This unusual relationship to morphologically different sporeforming organisms (the dif-

TABLE 6. DNA-DNA hybridization studies with *Peptostreptococcus micros*, *Peptostreptococcus parvulus*, *Peptostreptococcus anaerobius*, and Hare group IX, using the renaturation method

Strain	D value (%) with:				
	<i>Peptostreptococcus micros</i> DSM 20468 ^T	Hare group IX strains		<i>Peptostreptococcus parvulus</i> DSM 20469 ^T	<i>Peptostreptococcus anaerobius</i> DSM 20357
		NCTC 9811	NCTC 9821		
<i>Peptostreptococcus micros</i> DSM 20367	81	39	57	29	<25
<i>Peptostreptococcus micros</i> DSM 20468 ^T		38	55	30	<25
Hare group IX strain NCTC 9811			76	31	<25
Hare group IX strain NCTC 9821				26	<25
<i>Peptostreptococcus parvulus</i> DSM 20469 ^T					28

TABLE 7. DNA-rRNA hybridization studies of anaerobic gram-positive bacteria^a

Source of filter-bound DNA	$\Delta T_{m(e)}$ (°C) with [³ H]rRNA from:						
	<i>Peptostreptococcus asaccharolyticus</i> ATCC 14963 ^T	<i>Peptostreptococcus magnus</i> DSM 20362	<i>Peptostreptococcus prevotii</i> DSM 20358	<i>Peptostreptococcus parvulus</i> DSM 20469	<i>Clostridium lituseburense</i>	<i>Eubacterium tenue</i>	<i>Streptococcus sanguis</i>
<i>Peptostreptococcus asaccharolyticus</i> ATCC 14963 ^T	0 (73.5) ^b	8.3	11.1	12.1	17.6	ND ^c	ND
<i>Peptostreptococcus indolicus</i> ATCC 29427 ^T	3.5	9.5	11.7	ND	18.2	12.6	ND
<i>Peptostreptococcus asaccharolyticus</i> DSM 20364	5.6	8.2	8.7	10.8	14.2	ND	14.0
Hare group VIII strain NCTC 9820	5.5	7.8	9.1	ND	15.9	15.0	ND
<i>Peptostreptococcus magnus</i> DSM 20362	8.3	0 (78.8)	6.5	ND	15.3	9.4	ND
<i>Peptostreptococcus magnus</i> ATCC 14955	9.6	0.1	ND	11.3	17.7	10.0	14.8
Hare group IX strain NCTC 9811	10.6	8.2	11.5	ND	16.1	15.5	ND
Hare group IX strain NCTC 9821	9.0	7.7	9.6	ND	14.2	14.5	ND
<i>Peptostreptococcus prevotii</i> DSM 20358	10.1	9.0	0 (74.8)	12.4	ND	11.7	15.1
Hare group VIII strain NCTC 9810	9.5	7.7	2.3	ND	11.5	15.4	ND
Hare group I strain NCTC 9801	ND	7.4	2.6	7.8	16.8	13.6	ND
Hare group III strain NCTC 9814	8.6	7.4	3.7	ND	16.8	15.2	ND
<i>Peptostreptococcus parvulus</i> DSM 20469 ^T	12.9	13.8	16.2	0 (73.8)	ND	15.8	14.3
<i>Peptostreptococcus micros</i> DSM 20367	7.9	8.2	9.6	4.0	ND	12.3	15.3
<i>Clostridium lituseburense</i> ATCC 25759 ^T	13.0	9.8	12.5	ND	0 (79.1)	3.1	ND
<i>Eubacterium tenue</i> ATCC 25553 ^T	9.4	10.6	9.7	ND	4.4	0 (79.2)	ND
<i>Peptostreptococcus anaerobius</i> DSM 20357	10.8	10.7	11.0	ND	6.5	5.0	15.3
<i>Clostridium butyricum</i> DSM 552 ^T	14.2	12.2	16.2	ND	23.0	12.7	ND
<i>Streptococcus sanguis</i> DSM 20066	ND	ND	ND	ND	ND	ND	0 (77.5)
Hare group VIa strain NCTC 9806	11.8	10.8	10.1	11.8	13.2	ND	1.6

^a The preparations were incubated in double standard saline citrate containing 20% formamide at 50°C.

^b The numbers in parentheses are the temperatures at which 50% of the bound rRNA ($T_{m(e)}$) was eluted from the filters for the homologous hybrids.

^c ND, Not determined.

ference in G+C contents is about 8 mol%) has been confirmed by comparative sequence analyses of 16S rRNAs (37; W. Ludwig, Ph.D. thesis, Technical University of Munich, Munich, Federal Republic of Germany, 1981). The S_{AB} values (oligonucleotide similarities) of about 0.75 among these strains are equivalent to the values within the well-defined genus *Staphylococcus* (28). This surprising similarity of rRNAs between representatives of the clostridia and organisms which would not, if classical criteria were used, be regarded as belonging to the clostridia, is not an isolated case (16). *Clostridium butyricum*, as a representative species and the type species of the genus *Clostridium*, shows no relationship to *C. lituseburense* or any of the other strains examined in this study.

Our data support the relationship of the anaerobic aerotolerant species *Streptococcus intermedius* (21) to other members of the genus *Streptococcus* (e.g., *Streptococcus sanguis*). *Peptostreptococcus parvulus* also has been described as belonging to the genus *Streptococcus* on the basis of increased fermentative ability and production of lactic acid when growth is stimulated by Tween 80 (3). However, our hybridization data do not indicate any relationship at the genus level. DNA-rRNA hybridization studies showed a high $\Delta T_{m(e)}$ (11 to 14°C) between *Peptostreptococcus parvulus* DSM 20469 and *Streptococcus intermedius* NCTC 9806, as well as *Streptococcus sanguis* DSM 20066. *Peptostrep-*

tococcus parvulus also is not related to *Streptococcus pyogenes* NCTC 8198, the type species of the genus *Streptococcus* (R. Kilpper-Bälz, personal communication).

Peptococcus niger, the type species of the genus *Peptococcus*, was not included in our DNA-rRNA hybridization studies because of difficulties in preparing high-molecular-weight DNA or radioactively labeled rRNA. With a G+C content of about 50 mol%, this species is certainly not related to the other anaerobic, gram-positive cocci (G+C contents, 30 to 36 mol%). However, *Peptococcus niger* and *Peptostreptococcus parvulus* both possess the same type of murein and have similar G+C contents (Table 1). Further study will be necessary to determine the relatedness of these two species.

All of the strains of the different Hare groups which we examined can be assigned to existing species or genera. However, Hare group VIII was not uniform in terms of fermentation products (40) or hybridization data. On the other hand, Hare group I strain NCTC 9801 and group III strain NCTC 9814, which have a DNA homology value (D value) of 77%, should belong to one species (2). Together with Hare group VIII strain NCTC 9810 these two strains are related to *Peptostreptococcus prevotii* DSM 20358 [$\Delta T_{m(e)}$, 2 to 4°C]. Another strain of Hare group VIII (strain NCTC 9820), which has a D value of 54%, can be regarded as a subspecies of *Peptostreptococcus asaccharolyticus*. Both

TABLE 8. Diversity of murein structures of some closely related anaerobic cocci

Strain(s)	Murein structure			D value (%) ^a	$\Delta T_{m(e)}$ (°C) ^b
	Peptide subunit		Interpeptide bridge		
	Position 1	Position 3			
<i>Peptostreptococcus micros</i> DSM 20468 ^T	Gly	Orn	←D-Asp	38–57	ND ^c
Hare group IX strains NCTC 9811 and NCTC 9821	Gly	Lys	←D-Asp	38–57	ND
Hare group I strain NCTC 9801	Ala	Lys(Orn)	Lys-D-Glu	77	2–4
Hare group III strain NCTC 9814	Ala	Lys(Orn)	Gly ₅	77	2–4
<i>Peptostreptococcus prevotii</i> DSM 20358	Gly	Lys	D-Glu	25	2–4
Hare group VIII strain NCTC 9810	Gly	Lys	Gly-D-Asp	25	2–4
<i>Peptostreptococcus asaccharolyticus</i> DSM 20364	Ala	Orn	D-Glu	54	ND
Hare group VIII strain NCTC 9820	Gly	Lys	Gly-D-Asp	54	ND

^a DNA homology.^b See Table 7.^c ND, Not determined.

strains of Hare group IV are *Peptostreptococcus magnus* (Table 3), and the strains of group IX are related to *Peptostreptococcus micros* at the species or subspecies level.

Apart from the *Peptococcaceae*, relationships could be demonstrated with other families. Hare group VIIb strain NCTC 9819 is a strain of *Staphylococcus saccharolyticus*, an anaerobic member of the genus *Staphylococcus* (24, 25). Finally, Hare group VIa strain NCTC 9806 is related to *Streptococcus intermedius* ATCC 27335^T.

From our results and previous nucleic acid hybridization studies (11, 12, 23, 24), we concluded that the anaerobic, gram-positive cocci form a heterogenous group. The G+C contents of the DNAs of the type strains of *Peptococcus niger* (49 to 51 mol%) and *Peptostreptococcus parvulus* (44 to 46 mol%) differ significantly from those of other anaerobic, gram-positive cocci (27 to 35 mol%). However, the proposed transfer of *Peptostreptococcus parvulus* to the genus *Streptococcus* could not be confirmed by our results.

On the basis of differences in the G+C contents of the DNAs and the cellular fatty acid profiles, Ezaki et al. proposed to transfer *Peptococcus asaccharolyticus*, *Peptococcus indolicus*, *Peptococcus magnus*, and *Peptococcus prevotii* to the genus *Peptostreptococcus*. Our data, in particular the DNA-rRNA homology data, indicate the relatedness of at least some of the newly proposed members of the genus *Peptostreptococcus* (e.g., *Peptostreptococcus magnus* and *Peptostreptococcus prevotii*; *Peptostreptococcus asaccharolyticus* and *Peptostreptococcus indolicus*). However, other peptostreptococci, such as *Peptostreptococcus micros*, *Peptostreptococcus parvulus*, and *Peptostreptococcus anaerobius*, show $\Delta T_{m(e)}$ values in DNA-rRNA hybridization studies with the former group that are too high to justify their allocation to the same genus (35). This differentiation is also supported by a comparative sequence analysis of the 16S rRNAs of *Peptostreptococcus asaccharolyticus* ATCC 14963^T and *Peptostreptococcus micros* ATCC 23195 (formerly called *Peptococcus glycinophilus* ATCC 23195^T [4]). The S_{AB} value of 0.41 indicates that these two species are not related at the genus level (38). Final statements about the taxonomic liability of the genus *Peptostreptococcus* can be made only after DNA-rRNA hybridization studies with *Peptostreptococcus anaerobius* ATCC 27337^T, the type strain of the type species of the genus *Peptostreptococcus*, or, even better, after a comparative sequence analysis of the 16S rRNA of this organism.

The murein type of gram-positive bacteria is normally a valuable taxonomic marker (33). However, we found a striking variety of murein types in the anaerobic cocci examined in this study, even in closely related strains (Table 8). Although the substitution of ornithine by lysine between *Peptostreptococcus micros* and Hare group IX strains may be trifling, the variety of types within the *Peptostreptococcus prevotii* group is remarkable. Strains NCTC 9801 and NCTC 9814 obviously belong to one species (D value, 77%), but they have two completely different interpeptide bridges in their mureins, something that has never been observed before. Positions 1 and 3 of the peptide subunit are also different in the mureins of *Peptostreptococcus asaccharolyticus* DSM 20364 and NCTC 9820, which have 54% DNA homology. The primary structure of the murein is obviously a variable marker in some gram-positive anaerobic cocci. Therefore, care has to be taken in deducing relationships solely on the basis of murein types.

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