Numerical Taxonomy of *Streptococcus*

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A numerical taxonomic study of strains of *Streptococcus*, together with representatives of allied genera, showed 28 reasonably distinct phenons. The major areas, with their phenons, were: (a) enterococcal species group (*S. faecalis*, *S. faecium*, '*S. avium*' and a proposed new species '*S. gallinarum*'); (b) paraviridans species group (*S. bovis*, *S. equinus*, *S. salivarius*, '*S. casseliflavus*', *S. mutans*, *S. raffinolactis* and an unidentified Oral Group I); (c) lactic species group (*S. lactis* including *S. cremoris*); (d) thermophilic species group (*S. thermophilus*); (e) viridans species group (*S. mitis*, *S. sanguis*, a proposed new species '*S. oralis*' and '*S. milleri*'); (f) pyogenic species group (*S. agalactiae*, *S. pyogenes*, *S. equi*, '*S. equisimilis*' including '*S. zooepidemicus*', and a cluster of Lancefield Group B strains of human origin); (g) parapyogenic species group (*S. uberis*, '*S. dysgalactiae*', and a cluster of strains of Lancefield Groups R, S and T). Species of *Aerococcus*, *Gemella*, *Leuconostoc* and *Pediococcus* are very closely related to the streptococci.

INTRODUCTION

The genus *Streptococcus* is found in a wide variety of human, animal and plant habitats. Streptococci are important in the dairy industry, as pathogens of animals and man, and for their role in dental caries. The genus was proposed by Rosenbach (1884) and its history has been recently reviewed by Wilson & Miles (1975) and Jones (1978). The most important early papers were those of Andrewes & Horder (1906), Orla-Jensen (1919), Lancefield (1933) and Sherman (1937). In recent years there have been several numerical taxonomic analyses, leading to comprehensive study of large parts of the genus (Colobert & Blondeau, 1962; Carlsson, 1968; Colman, 1968; Seyfried, 1968; Jones *et al.*, 1972; Feltham, 1979), and these findings have been incorporated in varying degrees into taxonomic revisions (Deibel & Seeley, 1974; Jones, 1978).

The present paper is based on the study of Bridge (1981). It is an attempt to survey the entire genus, and therefore each species had to be restricted to rather few strains. The names of species that are not in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), and which are consequently not legitimate, are here placed in inverted commas.

METHODS

Strains. These are listed in Table 1. Strains were checked for purity and stored as suspensions in basal medium broth containing 15% (v/v) glycerol on beads at -76°C (Feltham *et al.*, 1978). Working cultures were maintained on Blood Agar Base No. 2 (Difco).

Basal media. Basal medium broth (BMB) contained (l^{-1}): proteose peptone (Difco), 15 g; NaCl, 5 g; yeast extract (Difco), 5 g; and liver digest (Oxoid), 2.5 g. Basal medium agar (BMA) was Blood Agar Base No. 2 (Difco).

Standard cultural conditions. Except when stated, all cultures were incubated at 37°C aerobically. Strains of *Gemella* and *Leuconostoc* were incubated at 25°C aerobically. The standard inoculum was one loopful of a 16 h BMB culture diluted to give the same turbidity as tube no. 2 on the MacFarland scale. When horse blood (Difco) was added, the final concentration was 7.5% (v/v).

Abbreviations: BMA, Basal medium agar; BMB, basal medium broth; ONPG, *o*-nitrophenyl- β -D-galactopyranoside; OTU, operational taxonomic unit; VP, Voges-Proskauer.

Table 1. Strains studied and their clustering (using the S_G coefficient) in the same order as Fig. 1

Phenon	Strain no.	Name as received	Lancefield group as received	Source* and collection no.	Comments†
1	PB1	<i>Streptococcus faecalis</i>	D	DJ W1	
	PB2	<i>S. faecalis</i>	D	DJ W48	TAL. NCTC 775, ATCC 19433 (GC 38.4)
	PB3	' <i>S. faecalis</i> subsp. <i>liquefaciens</i> '	D	DJ W2	CEN.
	PB4	' <i>S. faecalis</i> subsp. <i>zymogenes</i> '	D	DJ W3	
	PB79	' <i>S. faecalis</i> subsp. <i>zymogenes</i> '	D	RKAF K489	NCDO 1595, ATCC 10100
	PB126	' <i>S. faecalis</i> subsp. <i>liquefaciens</i> '	D	RKAF K525	Danish blue cheese, NCTC 8176
	PB128	' <i>S. faecalis</i> subsp. <i>liquefaciens</i> '	D	RKAF K534	Infant faeces, NCDO 591
	PB77	<i>S. faecalis</i>	D	RKAF K432	NCDO 1517, NCTC 6783
	PB129	<i>S. faecium</i>	D	RKAF K537	NCDO 583
2	PB85	' <i>S. faecalis</i> subsp. <i>liquefaciens</i> '	D	RKAF K454	Infant faeces, NCDO 611
	PB5	<i>S. faecium</i>	D	DJ W4	
	PB6	<i>S. faecium</i>	D	DJ W5	
	PB10	' <i>S. durans</i> '	D	DJ W11	Type† NCTC 8307, ATCC 19432, NCDO 596
	PB86	' <i>S. durans</i> '	D	RKAF K456	Type† NCTC 8307, ATCC 19432, NCDO 596 (GC 37.0)
	PB97	' <i>S. durans</i> '	D	RKAF K478	CEN. NCDO 502
	PB87	<i>S. faecium</i>	D	RKAF K457	TAL. NCTC 7171, NCDO 942 (GC 39.0)
	PB16	' <i>S. avium</i> '	Q	DJ W34	Deibel strain CC P1
	PB125	<i>S. faecalis</i>	D	RKAF K529	NCDO 1353
Ungrouped	PB140	<i>Streptococcus</i> sp.	D	RKAF K576	Human
	PB14	' <i>S. avium</i> '	Q	DJ W32	Deibel strain SS1
	PB15	' <i>S. avium</i> '	Q	DJ W33	CEN. Deibel strain Srep. A (GC 39.9)
	PB17	' <i>S. avium</i> '	Q	DJ W35	Deibel strain CAH2
	PB88	' <i>S. avium</i> '	Q	RKAF K461	Guthof strain E6844, NCTC 9938, ATCC 14025
	PB69	' <i>S. faecalis</i> subsp. <i>malodoratus</i> '	D	RKAF K415	Dutch cheese, NCDO 847
	PB18	<i>Streptococcus</i> sp.	D	EB F87/283	CEN. Chicken gut (GC 35.6)
	PB20	<i>Streptococcus</i> sp.	D	EB F87/275	Chicken gut
4	PB25	<i>Streptococcus</i> sp.	D	EB F87/296	Chicken gut
	PB29	<i>Streptococcus</i> sp.	D	EB F87/358	CEN. Chicken gut
	PB22	<i>Streptococcus</i> sp.	D	EB F87/277	Chicken gut
	PB24	<i>Streptococcus</i> sp.	D	EB F87/279	Chicken gut
	PB28	<i>Streptococcus</i> sp.	D	EB F87/344	Chicken gut
	PB19	<i>Streptococcus</i> sp.	D	EB F87/274	Chicken gut
	PB21	<i>Streptococcus</i> sp.	D	EB F87/276	PTS. Chicken gut, NCTC 11428 (GC 39.2)

Ungrouped	PB23	<i>Streptococcus</i> sp.	D	EB F87/278	Chicken gut
	PB27	<i>Streptococcus</i> sp.	D	EB F87/311	Chicken gut
	PB31	<i>Streptococcus</i> sp.	D	EB F87/362	Chicken gut
	PB33	<i>Streptococcus</i> sp.	D	EB F87/365	Chicken gut
	PB32	<i>Streptococcus</i> sp.	D	EB F87/364	Chicken gut
	PB34	<i>Streptococcus</i> sp.	D	EB F87/366	Chicken gut
	PB30	<i>Streptococcus</i> sp.	D	EB F87/361	Chicken gut
	PB26	<i>Streptococcus</i> sp.	D	EB F87/307	Chicken gut
	PB53	<i>S. faecium</i>	D	DJ W88	Motile, ATCC 14432, NCIB 9645
	PB152	<i>Streptococcus</i> sp.	LUM		Rat eye
5	PB153	<i>Streptococcus</i> sp.	D	NCTC 4725	Motile
	PB154	<i>Streptococcus</i> sp.	K	NCTC 10232	
	PB156	<i>Streptococcus</i> sp.	L	NCTC 10238	Dog
	PB109	' <i>Aerococcus catalasicus</i> '		NCIB 9643	Clausen C9, medicine bottles, NCDO 1841
	PB110	' <i>Aerococcus catalasticus</i> '		NCIB 9642	Clausen C6, medicine bottles, NCDO 1840
	PB143	<i>S. salivarius</i>	K	RKAF K582	Human mouth
	PB169	<i>Pediotococcus acidilactici</i>		NCIB 7881	
	PB7	<i>S. bovis</i>	D	DJ W7	Barnes strain TMC 33
	PB80	<i>S. bovis</i>	D	RKAF K434	CEN. NCDO 1251 (GC 39.3)
	PB81	<i>S. bovis</i>	D	RKAF K439	Infant faeces, NCDO 599
6	PB78	<i>S. bovis</i>	D	RKAF K433	NCDO 964
	PB11	<i>S. equinus</i>	D	DJ W14	CEN. Smith ET1
	PB13	<i>S. equinus</i>	D	DJ W16	CEN. Smith G1
	PB12	<i>S. equinus</i>	D	DJ W15	Smith IT3
	PB83	<i>S. equinus</i>	D	RKAF K443	TAL. Horse faeces, ATCC 9812, NCDO 1037 (GC 35.6)
	PB9	<i>S. salivarius</i>	K	RKAF K485	TAL. ATCC 7073, NCTC 8618 (GC 38.9)
	PB175	<i>Streptococcus</i> sp.		CU MSS2	Human mouth, Group III
	PB176	<i>Streptococcus</i> sp.	K	CU KTS1	Human mouth, Group III
	PB189	<i>Streptococcus</i> sp.		CU LV71	Human mouth, Group IV
	PB92	' <i>Streptococcus</i> sp. (MG)'		RKAF K465	NCTC 8037
7	PB99	<i>S. salivarius</i>	K	RKAF K483	' <i>S. hominis</i> ', NCTC 7366
	PB100	<i>S. salivarius</i>	K	RKAF K484	CEN. ' <i>S. hominis</i> ' (Type 1 <i>salivarius</i>), NCTC 8606
	PB51	' <i>S. faecium</i> subsp. <i>casseliflavus</i> '	D	DJ W86	Plant leaf, ATCC 25788
	PB52	' <i>S. faecium</i> subsp. <i>casseliflavus</i> '	D	DJ W87	Plant leaf, ATCC 25789 (GC 39.9)
	PB59	' <i>S. sobrinus</i> '		RKAF K597	CEN. SL1, type strain (Coykendall, 1977)
	PB82	<i>S. equinus</i>	D	RKAF K441	NCDO 1090
	PB8	<i>S. bovis</i>	D	DJ W9	Barnes strain TMC 101
	PB58	<i>S. rattus</i>		RKAF K592	CEN. TAL. Rat caries, FA1, ATCC 19645 (GC 38.8)
	PB76	<i>S. mutans</i>		RKAF K431	TAL. Carious dentine, ATCC 25175, NCTC 10449

Table 1 (*continued*)

Phenon	Strain no.	Name as received	Lancefield group as received	Source* and collection no.	Comments†
10	PB198	<i>Streptococcus</i> sp.		NCDO 2113	Raw carrot
	PB202	<i>Streptococcus</i> sp.		NCDO 2112	Raw carrot (GC 40.4)
	PB199	<i>S. raffinolactis</i>	N	NCDO 617	TAL. CEN. Milk (GC 39.8)
	PB200	<i>S. raffinolactis</i>	N	NCDO 619	Milk
11	PB171	<i>Streptococcus</i> sp.		CU OPK1	Human mouth, Carlsson Group II
	PB172	<i>Streptococcus</i> sp.		CU KPSK2	Human mouth, Carlsson Group II (GC 38.1)
	PB173	<i>Streptococcus</i> sp.		CU HPK1	Human mouth, CEN. Carlsson Group II
	PB174	<i>Streptococcus</i> sp.	K	CU OTS1	Human mouth, Carlsson Group II
12	PB48	<i>S. lactis</i>	N	DJ W59	TAL. NCTC 6681, NCDO 604, ATCC 19435
	PB93	<i>S. lactis</i>	N	RKAF K466	TAL. NCTC 6681, NCDO 604, ATCC 19435 (GC 35.5)
	PB94	<i>S. lactis</i>	N	RKAF K467	NCDO 496, ATCC 11494
	PB95	<i>S. cremoris</i>	N	RKAF K471	TAL. NCDO 607, ATCC 19257 (GC 34.1)
	PB96	<i>S. cremoris</i>	N	RKAF K472	Cheese starter, NCDO 500
	PB197	<i>S. cremoris</i>	N	LUM C616	TAL. NCDO 607, ATCC 19257
	PB130	<i>'S. lactis</i> subsp. <i>diacetylactis</i> '	N	RKAF K545	NCDO 184, NCIB 8765
	PB131	<i>'S. lactis</i> subsp. <i>diacetylactis</i> '	N	RKAF K547	Cheese starter, NCDO 1007
	PB193	<i>'S. lactis</i> subsp. <i>diacetylactis</i> '	N	NCDO 176	Type strain of <i>'S. diacetylactis</i> '§
	PB194	<i>'S. lactis</i> subsp. <i>alactosus</i> '	N	NCDO 802	CEN. NRRL B-1232
	PB196	<i>S. lactis</i>	N	NCIB 10769	ATCC 7963
	PB195	<i>'S. lactis</i> subsp. <i>diacetylactis</i> '	N	LUM C615	NCIB 10493, ATCC 11007 (GC 34.8)
13	PB107	<i>Aerococcus viridans</i>		NCIB 9206	<i>'Pediococcus homari</i> ', NCMB 680 (GC 39.9)
	PB148	<i>Streptococcus</i> sp.		LUM T58	CEN. (GC 40.2)
	PB149	<i>Streptococcus</i> sp.		LUM T128	
Ungrouped	PB201	<i>Streptococcus</i> sp.		NCDO 2128	
14	PB35	<i>S. thermophilus</i>		DJ W36	NCDO 489
	PB36	<i>S. thermophilus</i>		DJ W37	TAL. Pasteurized milk, NCDO 573
	PB37	<i>S. thermophilus</i>		DJ W38	Pasteurized milk, NCDO 574
	PB38	<i>S. thermophilus</i>		DJ W39	CEN. Pasteurized milk, NCDO 575
	PB39	<i>S. thermophilus</i>		DJ W40	
	PB84	<i>S. thermophilus</i>		RKAF K446	TAL. Pasteurized milk, NCDO 573
Ungrouped	PB124	<i>'S. cremoris</i> subsp. <i>alactosus</i> '		RKAF K521	Lactose negative, NCDO 1032
	PB151	<i>Streptococcus</i> sp.	E	RKAF K591	Coykendall LM7 (Group E, Facklam, 1977)
	PB49	<i>Streptococcus</i> sp.	O	DJ W60	Human, NCTC 8029

15	PB71	<i>Streptococcus</i> sp.	N	RKAF K424	Frozen peas, NCDO 1871
	PB43	<i>Streptococcus</i> sp.	F	DJ W53	NCTC 5389
	PB170	<i>Streptococcus</i> sp.		LGH MR6/79	Human
	PB46	<i>Streptococcus</i> sp.	K	DJ W56	NCTC 5338
	PB89	<i>Streptococcus</i> sp.		RKAF K490	Human sputum, NCTC 3166
	PB101	<i>S. mitis</i>		RKAF K492	
	PB90	<i>Streptococcus</i> sp.	O	RKAF K462	Human, NCTC 8029
	PB91	<i>Streptococcus</i> sp.	O	RKAF K464	Human, NCTC 8033
	PB98	<i>S. mitis</i>		RKAF K491	CEN. Human sputum, NCTC 10712 (GC 38.9)
	PB150	<i>Streptococcus</i> sp.		LUM T132	
	PB67	<i>S. sanguis</i>	H	RKAF K412	TAL. Human endocarditis, NCTC 7863, ATCC 10556
	PB68	<i>S. sanguis</i>	H	RKAF K413	CEN. Human endocarditis, NCTC 7865, ATCC 10558 (GC 39.3)
	PB146	<i>S. sanguis</i>	H	RKAF K589	
	PB145	' <i>S. mitior</i> '		RKAF K587	Human
	PB102	<i>Streptococcus</i> sp.		RKAF K495	Human tonsil, NCTC 1080
16	PB115	' <i>S. suis</i> '		LUM D1029	Elliott PM1
	PB123	' <i>S. suis</i> '		RKAF K520	DJ W13, Elliott PM1
	PB47	<i>Streptococcus</i> sp.	M	DJ W58	NCTC 7760
	PB157	<i>Streptococcus</i> sp.	M	NCTC 10235	Dog
	PB177	<i>Streptococcus</i> sp.		CU KPE2	Human mouth, Group IB
	PB178	<i>Streptococcus</i> sp.		CU MVE1	Human mouth, Group IB
	PB179	<i>Streptococcus</i> sp.		CU KPF1	Human mouth, Group IB (GC 39.9)
	PB187	<i>Streptococcus</i> sp.	G	CU NT61	Human mouth, Group VB
	PB188	<i>Streptococcus</i> sp.		CU PT51	Human mouth, Group VB
	PB180	<i>Streptococcus</i> sp.		CU LPA1	Human mouth, Group IA
	PB182	<i>Streptococcus</i> sp.		CU LVG1	PTS. Human mouth, Group IA, NCTC 11427
	PB190	<i>Streptococcus</i> sp.		CU LV81	Human mouth, Group IV
	PB181	<i>Streptococcus</i> sp.		CU MPA1	Human mouth, Group IA
	PB185	<i>Streptococcus</i> sp.		CU NS51	Human mouth, Group VA
	PB186	<i>Streptococcus</i> sp.		CU OP51	Human mouth, Group VA
17	PB183	<i>Streptococcus</i> sp.		CU OS51	CEN. Human mouth, Group VA (GC 40.0)
	PB184	<i>Streptococcus</i> sp.		CU LV51	Human mouth, Group VA
	PB56	' <i>S. milleri</i> '		RKAF K476	Colman 7331, from Guthof, NCTC 10708
	PB57	' <i>S. milleri</i> '		RKAF K477	CEN. Colman 7180, from Guthof, NCTC 10709 (GC 34.1)
	PB144	' <i>S. milleri</i> '		RKAF K585	Human
	PB60	' <i>S. sobrinus</i> '		RKAF K598	Coykendall K 1R
	PB70	<i>S. pneumoniae</i>		RKAF K416	TAL. NCTC 7465 (GC 35.1)
	PB66	<i>S. sanguis</i>		RKAF K411	Human endocarditis, NCTC 7864, ATCC 10557
18	PB56	' <i>S. milleri</i> '		RKAF K476	Colman 7331, from Guthof, NCTC 10708
	PB57	' <i>S. milleri</i> '		RKAF K477	CEN. Colman 7180, from Guthof, NCTC 10709 (GC 34.1)
	PB144	' <i>S. milleri</i> '		RKAF K585	Human
	PB60	' <i>S. sobrinus</i> '		RKAF K598	Coykendall K 1R
	PB70	<i>S. pneumoniae</i>		RKAF K416	TAL. NCTC 7465 (GC 35.1)
	PB66	<i>S. sanguis</i>		RKAF K411	Human endocarditis, NCTC 7864, ATCC 10557
	PB56	' <i>S. milleri</i> '		RKAF K476	Colman 7331, from Guthof, NCTC 10708
	PB57	' <i>S. milleri</i> '		RKAF K477	CEN. Colman 7180, from Guthof, NCTC 10709 (GC 34.1)
	PB144	' <i>S. milleri</i> '		RKAF K585	Human
	PB60	' <i>S. sobrinus</i> '		RKAF K598	Coykendall K 1R
	PB70	<i>S. pneumoniae</i>		RKAF K416	TAL. NCTC 7465 (GC 35.1)
	PB66	<i>S. sanguis</i>		RKAF K411	Human endocarditis, NCTC 7864, ATCC 10557
	PB56	' <i>S. milleri</i> '		RKAF K476	Colman 7331, from Guthof, NCTC 10708
	PB57	' <i>S. milleri</i> '		RKAF K477	CEN. Colman 7180, from Guthof, NCTC 10709 (GC 34.1)
	PB144	' <i>S. milleri</i> '		RKAF K585	Human

Table 1 (*continued*)

Phenon	Strain no.	Name as received	Lancefield group as received	Source* and collection no.	Comment†
19	PB161	<i>Gemella haemolysans</i>		NCTC 10243	TAL. ATCC 19256
	PB165	<i>Leuconostoc lactis</i>		NCDO 533	CEN. TAL. NRRL B-3471
	PB162	<i>Leuconostoc paramesenteroides</i>		NCDO 803	TAL. Wine, ATCC 23279
	PB163	<i>Leuconostoc oenos</i>		NCDO 1674	TAL. Cheese starter, ATCC 19254
	PB164	<i>Leuconostoc cremoris</i>		NCDO 543	NCDO 800
	PB166	<i>Leuconostoc dextranicum</i>		NCIB 2706	NRRL B-1142
	PB167	<i>Leuconostoc mesenteroides</i>		NCIB 3351	
Ungrouped	PB168	<i>Pediococcus halophilus</i>		NCIB 9735	NCDO 1872,
20	PB40	<i>S. agalactiae</i>	B	DJ W44	TAL. NCTC 8181, ATCC 13813 (GC 36.6)
	PB72	<i>S. agalactiae</i>	B	RKAF K426	CEN. TAL. NCTC 8181, ATCC 13813
	PB75	<i>S. agalactiae</i>	B	RKAF K430	NCDO 1520
	PB73	<i>S. agalactiae</i>	B	RKAF K428	NCDO 1352
	PB74	<i>S. agalactiae</i>	B	RKAF K429	NCDO 1334, NCTC 8183
	PB54	<i>S. pyogenes</i>	A	RKAF K400	TAL. Human throat, NCTC 8198, ATCC 12344 (GC 35.8)
	PB55	<i>S. pyogenes</i>	A	RKAF K403	CEN. Human puerperal sepsis, NCTC 8191
21	PB61	<i>S. pyogenes</i>	A	RKAF K401	CEN. Human throat, NCTC 2218
	PB62	<i>S. pyogenes</i>	A	RKAF K405	Human throat, NCTC 8316
	PB64	<i>S. pyogenes</i>	A	RKAF K407	Human, NCTC 4001
	PB65	<i>S. pyogenes</i>	A	RKAF K410	Human throat, NCTC 8302
	PB41	<i>S. equi</i>	C	DJK W45	CEN. TAL. NCTC 9682 (GC 39.3)
	PB63	<i>S. equi</i>	C	RKAF K406	CEN. TAL. NCTC 9682
	PB44	<i>Streptococcus</i> sp.	G	DJ W54	Human lymphangitis, NCTC 8547
23	PB103	' <i>S. equisimilis</i> '	C	RKAF K496	CEN. NCTC 5371 (GC 37.3)
	PB104	' <i>S. equisimilis</i> '	C	RKAF K499	NCTC 7136
	PB132	' <i>S. equisimilis</i> '	C	RKAF K549	Horse vagina, NCTC 6179
	PB120	' <i>S. zooepidemicus</i> '	C	RKAF K511	Mouse septicaemia, NCTC 6176
	PB121	' <i>S. zooepidemicus</i> '	C	RKAF K514	NCTC 7023
	PB105	' <i>S. equisimilis</i> '	C	RKAF K500	Human vagina, NCTC 8543
	PB155	<i>Streptococcus</i> sp.	L	NCTC 10321	(GC 38.1)
Ungrouped	PB45	<i>Streptococcus</i> sp.	H	DJ W55	NCTC 7868
	PB142	<i>Streptococcus</i> sp.	G	RKAF K579	Human

24	PB127	<i>Streptococcus</i> sp.	B	RKAF K528	Human puerperal sepsis, NCTC 9412
	PB139	<i>Streptococcus</i> sp.	B	RKAF K573	Human
	PB141	<i>Streptococcus</i> sp.	B	RKAF K578	Human
	PB133	<i>Streptococcus</i> sp.	B	RKAF K550	Human
	PB134	<i>Streptococcus</i> sp.	B	RKAF K551	Human
	PB137	<i>Streptococcus</i> sp.	B	RKAF K563	CEN. Human (GC 33-18)
	PB138	<i>Streptococcus</i> sp.	B	RKAF K567	Human
	PB147	<i>Streptococcus</i> sp.	B	RKAF K590	Human
	PB135	<i>Streptococcus</i> sp.	B	RKAF K558	Human
	PB136	<i>Streptococcus</i> sp.	B	RKAF K560	Human
Ungrouped	PB42	' <i>S. infrequens</i> '	E	DK W52	Low acid producer, NCTC 5385
	PB122	<i>Streptococcus</i> sp.	E	RKAF K519	Pig abscess, NCDO 603
	PB191	<i>Streptococcus</i> sp.	M	NCTC 1233	Dog, SHC/200
25	PB50	<i>S. uberis</i>		DJ W62	TAL. NCTC 3858, ATCC 19436, NCDO 2038
	PB118	<i>S. uberis</i>		RKAF K506	CEN. TAL. NCTC 3858, ATCC 19436, NCDO 2038, (GC 36-4)
	PB119	<i>S. uberis</i>		RKAF K510	Bovine mastitis, N29, NCTC 4674, NCDO 2041
26	PB106	' <i>S. dysgalactiae</i> '	C	RKAF K501	NCDO 1356
	PB117	' <i>S. dysgalactiae</i> '	C	RKAF K505	CEN. Bovine mastitis, NCTC 4669 (GC 37-2)
	PB116	' <i>S. dysgalactiae</i> '	C	RKAF K503	NCDO 1358
Ungrouped	PB158	<i>Streptococcus</i> sp.	P	NCTC 9824	ATCC 14024
27	PB159	<i>Streptococcus</i> sp.	R	NCTC 10234	Pig, 735
	PB160	<i>Streptococcus</i> sp.	S	NCTC 10237	CEN. Pig, Field 428 (GC 40-2)
	PB192	<i>Streptococcus</i> sp.	T	NCTC 10446	Pig brain, T639
28	PB108	<i>Pediococcus pentosaceus</i>		NCIB 8106	
	PB111	<i>Pediococcus acidilactici</i>		NCIB 6990	CEN. ATCC 8042, NCDO 521
	PB113	<i>Pediococcus halophilus</i>		NCIB 9477	
	PB112	<i>Aerococcus viridans</i>		NCIB 9208	NCTC 7766
	PB114	' <i>Pediococcus cerevisiae</i> '		NCIB 10563	Slime producer

* Sources: ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; CU, Professor J. Carlsson, Department of Oral Microbiology, University of Umea, Umea, Sweden; DJ, Dr D. Jones, Department of Microbiology, Leicester University, U.K.; EB, Dr E. Barnes, Food Research Institute, Norwich, U.K.; LGH, Leicester General Hospital, Leicester, U.K.; LUM, Microbiology Department, Leicester University, U.K.; NCDO, National Collection of Dairy Organisms, National Institute for Research in Dairying, Shinfield, Reading, U.K.; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, U.K.; NCMB, National Collection of Marine Bacteria, Torry Research Station, Aberdeen, U.K.; NCTC, National Collection of Type Cultures, Public Health Laboratory Service, Colindale, London, U.K.; RKAF, Dr R. K. A. Feltham, Microbiology Department, Leicester University, Leicester, U.K.

† Abbreviations: TAL, type strain in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980); PTS, proposed type strain (Bridge & Sneath, 1982); GC, mol % guanine plus cytosine found in this study; CEN, centrotypic or joint centrotypic; CU, groups of strains marked CU are those in Carlsson (1968).

§ National Collection of Dairy Organisms (1974).

Colonial and cellular morphology. Strains grown on BMA with horse blood for 24 h were examined for colonial features. Haemolysis was read at 16 h; complete lysis was recorded as β -haemolysis and greening (with or without partial lysis) as α -haemolysis. The CAMP test was performed as described by Christie *et al.* (1944) using washed sheep red cells (Gibco, 7.5%, v/v) and *Staphylococcus aureus* (NCTC 7428). Results were recorded after 24 h. Heat-fixed films of 24 h BMB cultures were stained by Gram's stain (Cowan & Steel, 1974).

Growth at different temperatures. Strains were grown in BMB for 24 d at 4 °C; 10 d at 10 °C; 24 h at 25 °C; and 16 h at 45 °C, and growth was recorded as visible turbidity.

Tolerance to inhibitory compounds and heat. Growth was observed as visible turbidity in BMB containing 3, 4 and 6.5% (w/v) NaCl after 24 h. Growth with 10 and 40% (w/v) ox-bile (Oxoid), crystal violet (0.02 and 0.04 g l⁻¹), sodium azide (1 g l⁻¹) and thallos acetate (1 g l⁻¹) was observed by streaking strains on BMA with horse blood after 24 h incubation. Resistance to heat was determined by holding 10 ml amounts of inoculated BMB at 60 °C and plating out at 1, 15, 60 and 120 min on to BMA with horse blood. The plates were inspected for growth after 24 h. Although the result after 1 min heating is not very reliable because of the time needed for the tubes to heat up, it provided an indication of the most heat-sensitive strains. Bile solubility was observed on 24 h cultures in BMB. To these was added sterile ox-bile to give 10% (w/v), and the tubes were examined for clearing after 2 h at room temperature followed by 16 h at 4 °C. The method of Whittenbury (1965) was used for testing growth on acetic acid-acetate agar at pH 5.4. Growth on MacConkey agar (Oxoid) and TCBS agar (Oxoid) was observed on plate cultures after 48 h incubation. The production of acid on these media (red and yellow colonies, respectively) was also recorded. Growth at pH 9.6 was observed by the method of Chesbro & Evans (1959).

Biochemical tests. Methyl red and Voges-Proskauer (VP) tests were performed on BMB containing 0.5% (w/v) K₂HPO₄ and 0.5% (w/v) glucose. Cultures were tested after 3 and 7 d with methyl red for acid production. For the VP test, 40% (w/v) NaOH and 5% (w/v) α -naphthol were added: a deep red colour after 2 h was a positive reaction. Methyl red was also tested on the API 50E gallery (API Ltd, Farnborough, Surrey) and read after 24 h according to the manufacturer's instructions.

Oxidase and catalase were tested by the methods of Cowan & Steel (1974) on 16 h cultures grown on BMA. Deoxyribonuclease activity was observed after 48 h by the method of Smith *et al.* (1969) using DNAase agar (Difco) with 0.1% (w/v) yeast extract (Difco). This test was also performed on the API 50E gallery and read after 48 h. Phosphatase activity was examined by the method of Cowan & Steel (1974) using BMA containing 0.01% (w/v) phenolphthalein phosphate: plates were flooded with ammonia solution after 24 h. Urease activity was examined after 5 d by the modification of Christensen's medium used by Cowan & Steel (1974). The ONPG test for β -D-galactosidase activity (Cowan & Steel, 1974) was observed after 24 h.

Aesculin hydrolysis was detected by a dark colouration after 24 h on horse blood BMA containing 0.05% (w/v) ferric ammonium citrate and 0.1% (w/v) aesculin. This test was also performed on the API 50E gallery and read after 48 h according to the manufacturer's instructions. Starch hydrolysis was detected on BMA containing 0.5% (w/v) soluble starch: plates were flooded with Gram's iodine after 7 d incubation to stain unhydrolysed starch. Hydrolysis of hippurate was tested after 4 d by method (1) of Cowan & Steel (1974). D-Arginine hydrolysis (Cowan & Steel, 1974) was tested with Nessler's reagent to detect ammonia after 48 h. The same method was used for serine hydrolysis, with L-serine substituted for D-arginine. Casein hydrolysis was detected by the method of Cowan & Steel (1974) using one part of Skim Milk medium (Difco) mixed with two parts of double-strength BMA. The plates were examined for clearing after 3 d. Gelatin liquefaction was tested by method (2) of Cowan & Steel (1974). Readings were taken (after first cooling for 1 h at 4 °C) at 14 d.

Indole production was tested after 48 h in BMB with Kovac's reagent (Cowan & Steel, 1974). Dextran production was observed in double-strength BMB containing 5% (w/v) sucrose. Dextran was flocculated by the addition of ethanol (Cowan & Steel, 1974). Production of levan was examined by the method of Cowan & Steel (1974) on BMA containing 0.5% (w/v) sucrose and 0.5% (w/v) horse serum after 4 d incubation. The production of hydrogen peroxide was observed by the method of Whittenbury (1975) as dark colouration around colonies on heated blood agar (BMA containing horse blood held at 54 °C for 1 h) with 0.1% (w/v) *o*-dianisidine. The production of hydrogen sulphide from thiosulphate was tested by the method of Feltham (1975) by stab inoculating tubes of medium consisting of (l⁻¹): beef extract (Difco), 3 g; tryptone (Difco), 30 g; Na₂S₂O₃ · 5H₂O, 50 mg; cysteine HCl, 0.2 g; NaCl, 5 g; ferric ammonium citrate, 0.5 g; agar, 3 g. The medium was observed over 5 d for blackening.

Decarboxylation reactions were observed after 4 d for lysine, ornithine and arginine by the method of Falkow (1958). Decarboxylation medium consisted of (l⁻¹): yeast extract (Difco), 3 g; Bacto peptone (Difco), 5 g; glucose, 1 g; and bromocresol purple (1.6%, w/v), 1 ml. Amino acids were added at 5 g l⁻¹, and 6 ml samples were layered with liquid paraffin. Arginine dihydrolase may contribute to positive results in the arginine medium.

BMA was used to test reduction of potassium tellurite (0.32 g l⁻¹), 2,3,5-triphenyltetrazolium chloride (1 g l⁻¹) and sodium selenite (0.1 g l⁻¹); the plates were incubated for 48 h. Reduction of tetrazolium and selenite was observed as red colonies, reduction of tellurite was observed as black colonies; strains showing no growth were recorded as negative. Reduction of methylene blue and janus green B was observed in skim milk medium

containing either methylene blue (0.2 g l^{-1}) or janus green B (0.08 g l^{-1}). Tubes were observed for reduction after 16 h. Acid and clot production and discolouration of litmus was examined in litmus milk medium (Oxoid) over 7 d. Reduction of nitrate and nitrite in BMB containing either 0.1% (w/v) KNO_3 or 0.001% (w/v) NaNO_2 with Durham tubes was tested by the method of Cowan & Steel (1974). Nitrate was observed after 5 d (negative tubes were checked with zinc dust) and nitrite after 7 d, when gas production was also recorded.

The final pH after 7 d incubation in BMB with added glucose (5 g l^{-1}) was measured by a digital pH meter. Ethanol oxidation was tested as described by Skerman (1969): BMB containing 5% (v/v) ethanol was inoculated and incubated for 48 h. Acid production was detected by adding bromocresol purple. Utilization of citrate, malonate and acetate, and attack on mucate, gluconate and pectate, were determined on API 50E galleries read after 48 h in accordance with the manufacturer's instructions.

Further enzyme activities were investigated using the APIzym (API Ltd) system. Overnight cultures on BMA with horse blood were suspended in sterile distilled water to give a turbidity between that of tubes no. 5 and 6 on the MacFarland scale and added to the cupules. After 4 h at 37°C , the test reagent, made up according to the manufacturer's instructions, was added. The galleries were left for 30 s under intense light and then scored according to the API colour chart. Washings from uninoculated plates were used as controls; they gave negligible reactions. Lipase and tetrathionate reductase were determined in API 50E galleries according to the manufacturer's instructions and read after 48 h.

Production of acid from carbohydrates. API 50E galleries were used for 39 carbohydrate fermentations (Table 3). Growth from overnight cultures on BMA with horse blood was emulsified in API suspending medium to give a suspension with the same turbidity as tube no. 2 on the MacFarland scale. Cupules were inoculated with bacterial suspensions and the galleries were read at 24 and 48 h; the maximum acidity was recorded according to the manufacturer's instructions.

Antibiotic sensitivity. Sensitivity to antibiotics was examined on BMA with horse blood plates poured to a depth of 5 mm. A loopful of growth from a 24 h agar culture was emulsified in 2.5 ml sterile physiological saline and poured on to each plate. The excess liquid was removed with a sterile Pasteur pipette and the plates were allowed to stand for 30 min before antibiotic discs were placed on the surface. The plates were incubated at 37°C for 24 h. Growth within less than 2 mm of the disc was recorded as resistance. The antibiotics (Multodisks, Oxoid) and the quantity per disc were: penicillin G, 5 units; sulphafuroxazole, $500 \mu\text{g}$; ampicillin, $25 \mu\text{g}$; erythromycin, $10 \mu\text{g}$; novobiocin, $30 \mu\text{g}$; oleandomycin, $10 \mu\text{g}$; furazolidone, $100 \mu\text{g}$; carbenicillin, $10 \mu\text{g}$; nalidixic acid, $30 \mu\text{g}$; nitrofurantoin, $200 \mu\text{g}$; tetracycline, $50 \mu\text{g}$; chloramphenicol, $50 \mu\text{g}$; chlortetracycline, $50 \mu\text{g}$; oxytetracycline, $50 \mu\text{g}$.

Numerical taxonomy. Computing was performed on the University of Nottingham's ICL 1900 computer and the University of Leicester's CDC CYBER 73 computer using computer programs written by M. J. Sackin and associates of the Microbiology Department, Leicester University.

The $t \times n$ matrix was composed of 202 strains and 136 characters after excluding 21 invariant characters (see Table 3). Two-state characters were coded 0 for negative and 1 for positive. The characters from the API 50E and the APIzym strips were coded from 0 for negative to 5 for a strong positive reaction. Antibiotic sensitivity was recorded as positive.

The similarity coefficients used were the Gower coefficient (S_G), the Simple Matching coefficient (S_{SM}) and the Pattern Difference (D_P). Cluster analysis was by average linkage (UPGMA) and single linkage. The vigour coefficient of each strain was also calculated. Details of these methods are given in Sneath & Sokal (1973). For D_P and S_{SM} the multistate characters were converted to binary ones; for the API 50E results 0–2 was scored as negative and 3–5 as positive; for the APIzym results 0–1 was scored as negative and 2–5 as positive. Test reproducibility was estimated by limited replication of tests, and by duplicating 20 strains, whose PB numbers were 1, 14, 35, 40, 50, 100, 132, 144, 150 and 192–202. The duplicates were not used in the numerical taxonomy because the number of strains would then have exceeded the capacity of the computer. Test error was calculated by formula 1 in Sneath & Johnson (1972). Cell arrangement and sensitivity to certain additional antibiotics were very poorly reproducible, and these characters were therefore omitted.

Other calculations. The overlap statistics of phenons were calculated by the computer program OVCLUST (Sneath, 1979a). This used the number of operational taxonomic units (OTU) in each subphenon and the inter- and intra-centroid distances, d [based on the relation $d = (1 - S_{SM})^{1/2}$] to calculate an index of overlap (V_G) that was compared by a non-central t -test to a given critical level of overlap (V_{crit}). The level of overlap expected for a rectangular distribution (Sneath, 1977) was used as a critical level of overlap to compare all pairs of sulphenons.

A program, DIACHAR (Sneath, 1980a), was used to list the most diagnostic properties of the phenons, and the results form the basis of the comments on the salient phenon characteristics. The program CHARSEP (Sneath, 1979b) was used to calculate VSP separation indices for all tests used in the numerical taxonomy. The 60 tests that showed the highest separation values were used to construct an identification matrix. This was tested by the programs MOSTTYP (Sneath, 1980b) and OVERMAT (Sneath, 1980c). MOSTTYP showed the best identification score that could be obtained by the most typical representative of each taxon. OVERMAT

calculated overlap statistics for each taxon in the matrix and these results were expressed as for the OVCLUST calculations.

The principal coordinates analysis was made by the method of Gower (1966) from the taxonomic distances, d , between the centroids of the phenons (Table 2) using a program written by M. J. Sackin.

Cophenetic discrepancies are the contributions made by each strain to the distortion between (1) the similarities in the similarity matrix and (2) the corresponding values implied by the phenogram. These were measured by:

$$\Delta_j = \left[\sum_{k=1}^t (S_{jk} - C_{jk})^2 \right]^{\frac{1}{2}}$$

where S_{jk} and C_{jk} are the similarity between OTUs j and k in the similarity matrix and in the phenogram respectively. Further details are given in Sneath & Sokal (1973) and Sneath *et al.* (1981).

Mole percent GC. DNA was extracted and purified by the method of Garvie (1976), with addition of a final dialysis against saline citrate buffer, and melting point determinations were made in saline citrate buffer as described there. The denaturation curves were followed using a Beckman Model 35 spectrophotometer with a Stanton Redcroft Linear Temperature Variable Rate Programmer and an Edale Thermistor thermometer with a Grants Instruments temperature probe. The steepest point of the curve was taken as T_m , and the mol % GC obtained as $(T_m - 69.4) \times 2.44$ (De Ley, 1970).

RESULTS

Test reproducibility

The S_{SM} values between the replicates of the 20 duplicated strains had mean and standard deviation of $95.04 \pm 1.94\%$. This corresponds to a test error of 2.55%. After the work was completed, nine pairs of strains that had been carried through independently were found to derive from the same nine isolates which had come through different sources (Table 1). These pairs had mean and standard deviation of S_{SM} of 89.52 and 2.96%, corresponding to 5.55% test error, which is still acceptable.

Main cluster analysis

The different cluster analyses gave similar results. The taxonomy described here is based on the well-tried UPGMA method using the S_G coefficient (Fig. 1). The other analyses are briefly described later.

Twenty-eight phenons were recognized which seemed reasonably distinct. Where type strains were included these phenons were named accordingly, but the work was carried out before the Approved Lists (Skerman *et al.*, 1980) were available, and therefore some type strains were not examined. The phenons fell into a number of main areas which are described below. A small number of strains were ungrouped.

Main areas. The traditional main areas of the genus are seen in Fig. 1, though some have been subdivided. There are also major groups representing the other genera studied.

The enterococcal species group corresponds to the traditional enterococci, and this major section of the genus is well separated from the rest.

The traditional viridans streptococci can be seen in Fig. 1 to occur in several parts of the phenogram. One section contains *S. salivarius* and similar species; it is referred to as the paravidans species group. Adjoining this is the lactic species group, followed by one containing *S. thermophilus* that is called the thermophilic species group. Between the last two is an ill-defined area referred to as the aerococcal group.

The next major branch of the diagram starts with classical viridans streptococci such as *S. mitis*; this is called the viridans species group. The position of the pneumococci is unfortunately not clear. The strains of *S. pneumoniae* proved difficult to study, and full data were only obtained on one strain. The strain came in this area and is marked in Fig. 1 with an indication that it represents what may be a separate main area, the pneumococcal species group. Adjoining this is an area referred to as the leuconostoc group.

Fig. 1. Simplified UPGMA phenogram based on S_G . The single strains may be identified by referring to the ungrouped strains in Table 1. The cophenetic correlation was 0.711.

Table 2. *Parameters of phenons and intracentroid distances*

The table is based on the form of data in which all scores have been converted to positive and negative (see Methods: *Numerical taxonomy*). The entries in the body of the table are intercentroid taxonomic distances, from which Fig. 2 was derived. The mean vigour of each phenon is the mean proportion of characters that have positive states, averaging over all the strains in the corresponding phenon: s.d. indicates the standard deviations of these proportions. The mean distance of strains of a phenon to their own centroid, $\bar{d}_j$, is calculated as $\sqrt{(\sum d_{ij}^2/(t_j - 1))}$, where d_{ij}^2 is the squared distance of a strain to the centroid of its own phenon, and t_j is the number of strains in phenon j .

Phenon													
1	0.0												
2	0.287	0.0											
3	0.354	0.346	0.0										
4	0.366	0.375	0.326	0.0									
5	0.436	0.386	0.462	0.441	0.0								
6	0.472	0.405	0.483	0.507	0.301	0.0							
7	0.425	0.385	0.421	0.428	0.264	0.320	0.0						
8	0.412	0.343	0.411	0.428	0.322	0.350	0.321	0.0					
9	0.432	0.424	0.450	0.436	0.310	0.390	0.306	0.336	0.0				
10	0.427	0.420	0.440	0.434	0.361	0.391	0.287	0.347	0.297	0.0			
11	0.487	0.461	0.485	0.479	0.374	0.410	0.306	0.394	0.337	0.350	0.0		
12	0.355	0.334	0.418	0.435	0.311	0.357	0.267	0.337	0.346	0.290	0.390	0.0	
13	0.416	0.394	0.475	0.470	0.352	0.422	0.298	0.351	0.399	0.361	0.359	0.289	0.0
14	0.510	0.445	0.483	0.516	0.382	0.394	0.350	0.375	0.452	0.442	0.431	0.410	0.417
15	0.491	0.440	0.501	0.524	0.351	0.375	0.307	0.339	0.403	0.378	0.411	0.328	0.320
16	0.502	0.417	0.512	0.530	0.376	0.392	0.353	0.372	0.417	0.411	0.397	0.369	0.355
17	0.514	0.465	0.502	0.504	0.381	0.425	0.310	0.377	0.394	0.400	0.408	0.386	0.369
18	0.487	0.471	0.538	0.548	0.398	0.423	0.334	0.408	0.396	0.402	0.417	0.376	0.407
19	0.534	0.483	0.517	0.521	0.427	0.444	0.369	0.415	0.452	0.427	0.389	0.390	0.398
20	0.482	0.472	0.523	0.548	0.439	0.480	0.396	0.439	0.477	0.439	0.498	0.387	0.431
21	0.522	0.504	0.562	0.579	0.447	0.498	0.375	0.421	0.466	0.408	0.465	0.395	0.420
22	0.544	0.502	0.584	0.577	0.422	0.455	0.428	0.439	0.462	0.453	0.493	0.399	0.448
23	0.429	0.442	0.491	0.478	0.366	0.436	0.354	0.384	0.413	0.368	0.443	0.300	0.376
24	0.384	0.417	0.460	0.471	0.369	0.415	0.339	0.365	0.420	0.381	0.435	0.290	0.326
25	0.444	0.440	0.465	0.448	0.389	0.444	0.354	0.388	0.407	0.393	0.409	0.349	0.368
26	0.486	0.479	0.503	0.479	0.399	0.475	0.391	0.394	0.414	0.383	0.393	0.388	0.407
27	0.493	0.474	0.531	0.497	0.377	0.416	0.350	0.401	0.377	0.397	0.401	0.377	0.387
28	0.384	0.356	0.400	0.433	0.374	0.413	0.341	0.390	0.424	0.411	0.381	-0.358	0.328
Phenon	1	2	3	4	5	6	7	8	9	10	11	12	13
No. of strains	10	6	4	17	4	4	7	4	3	4	4	12	3
Mean vigour	0.575	0.503	0.565	0.581	0.433	0.381	0.414	0.428	0.461	0.419	0.385	0.429	0.421
s.d.	0.026	0.026	0.014	0.013	0.036	0.003	0.025	0.010	0.013	0.045	0.052	0.043	0.033
$\bar{d}_j$	0.257	0.246	0.234	0.213	0.248	0.223	0.301	0.272	0.271	0.263	0.278	0.280	0.328

[illegible]

Table 3 *Characteristics of the phenons (percentage of strains positive)*

Test	Phenon ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>Colonial characters:</i>																
Colony diameter < 0.2 mm		0	0	0	0	25	0	0	0	100	50	75	8	0	17	17
Colony diameter 0.2-0.4 mm		30	83	75	77	50	100	100	100	0	50	25	83	100	83	67
Colony diameter > 0.4 mm		70	17	25	23	25	0	0	0	0	0	0	8	0	0	17
α -Haemolysis		40	83	100	100	50	25	67	75	0	0	25	67	67	67	83
β -Haemolysis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
CAMP test		10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Red colonies on MacConkey agar		100	100	75	65	0	0	0	25	0	0	0	17	0	0	0
Yellow colonies on TCBS agar		50	50	75	94	0	0	0	0	0	0	0	0	50	0	0
<i>Growth characters:</i>																
At 4 °C		10	100	0	6	0	0	0	0	0	0	0	0	0	0	0
At 10 °C		100	100	100	100	0	0	17	25	0	75	0	92	100	0	33
At 25 °C		100	100	100	100	100	100	100	100	67	100	25	100	100	100	83
At 45 °C		100	100	100	100	100	100	83	100	100	0	25	0	100	100	100
At pH 9.6		100	100	100	100	0	0	0	50	0	0	0	0	33	0	0
With 3% (w/v) NaCl		100	100	100	100	100	100	67	100	67	75	0	100	67	100	100
With 4% (w/v) NaCl		100	100	100	100	100	100	50	100	67	75	0	75	33	17	33
With 6.5% (w/v) NaCl		100	100	100	100	0	0	0	0	0	25	0	0	33	0	0
With 10% bile		100	100	100	100	100	100	33	100	100	100	0	100	33	100	83
With 40% bile		100	100	100	100	100	100	17	100	70	0	0	42	0	100	33
On acetic acid-acetate agar		0	0	100	100	25	0	17	0	0	0	0	0	0	0	0
With sodium azide		100	100	100	100	75	100	83	100	100	100	0	83	0	83	33
With 0.0002% (w/v) crystal violet		20	17	75	53	0	0	67	25	100	75	100	25	67	100	17
With 0.0004% (w/v) crystal violet		10	0	25	12	0	0	50	0	0	0	75	0	33	0	0
On MacConkey agar		100	100	100	100	0	0	0	25	0	0	0	17	0	0	0
On TCBS agar		100	83	100	94	0	0	0	0	0	0	0	17	50	0	0
With thallos acetate		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
<i>Survival at 60 °C for:</i>																
1 min		100	100	100	100	100	100	100	100	100	100	25	100	100	100	83
15 min		100	100	100	77	75	100	33	75	30	100	25	92	100	100	83
1 h		100	100	25	0	75	75	17	50	0	0	0	33	100	83	50
2 h		30	17	0	0	25	75	0	0	0	0	0	0	0	17	0
<i>Biochemical characters:</i>																
Methyl red		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Methyl red (API)		80	100	100	94	100	75	50	100	100	75	100	100	100	83	50
VP		100	83	100	100	100	0	67	100	70	0	100	42	100	17	0
Urease		10	0	0	0	0	0	17	0	0	0	0	8	33	0	0
ONPG		70	100	25	88	0	0	0	50	0	0	0	33	67	0	0
DNAase (plate)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DNAase (API)		10	0	0	0	0	25	0	0	0	0	0	0	0	0	17
Lipase		40	67	100	88	50	100	67	100	70	0	50	67	0	67	33
Phosphatase (plate)		90	0	100	100	0	0	0	0	0	0	0	8	33	33	17
Aesculin hydrolysis (plate)		100	100	100	100	100	100	100	100	100	100	100	75	100	33	0
Aesculin hydrolysis (API)		100	100	100	100	75	100	100	25	100	100	100	92	67	0	0
Arginine hydrolysis		100	100	25	0	0	0	17	75	33	0	0	67	100	17	83
Casein hydrolysis		50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gelatin liquefaction		40	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hippurate hydrolysis		90	0	0	12	0	0	0	0	0	25	0	50	100	0	50
Starch hydrolysis		0	0	25	77	100	100	83	25	33	50	100	8	100	83	83
H ₂ O ₂ production		10	33	75	82	25	50	17	25	0	0	0	0	67	0	33
H ₂ S production		0	0	0	0	0	0	0	0	50	0	0	0	0	0	0
Dextran production		0	0	0	0	0	0	17	0	33	0	0	0	33	0	17
Levan production		0	0	0	0	0	0	33	0	0	0	25	0	0	0	0
Arginine decarboxylase		80	100	0	12	75	25	0	50	0	0	25	92	100	0	17
Lysine decarboxylase		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithine decarboxylase		10	0	0	0	0	0	33	0	0	0	0	0	33	17	17
<i>Reduction of:</i>																
Janus green		100	67	100	100	75	0	67	0	30	50	50	100	33	100	0
Litmus		90	83	100	100	100	100	100	50	100	75	100	100	100	100	100
Methylene blue		100	67	25	0	0	0	33	0	70	0	25	75	100	0	100
Nitrate		0	17	0	47	0	0	0	50	70	0	0	8	0	0	17

Table 3 (continued)

Test	Phenon ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Nitrite		100	100	75	100	100	100	50	100	100	0	100	25	33	100	0
Selenite		100	100	100	100	0	0	100	25	0	100	50	92	67	83	67
Tellurite		100	50	50	18	0	0	0	25	0	0	0	67	0	0	0
Tetrathionate		70	100	100	82	100	100	67	100	70	50	0	42	0	67	17
Tetrazolium		90	50	100	100	100	0	83	0	100	25	50	83	67	100	67
Final pH <4.25		100	100	100	100	100	100	100	75	100	100	100	92	100	100	33
Final pH 4.25-4.75		0	0	0	0	0	0	0	25	0	0	0	8	0	0	67
Final pH >4.75		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clot in litmus milk		40	67	100	0	100	25	100	50	70	50	0	75	33	100	100
Oxidation of ethanol		10	0	25	0	0	0	0	0	33	0	0	8	0	0	17
Citrate utilization		50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malonate utilization		0	0	0	47	0	0	0	0	0	0	0	0	0	0	0
<i>Enzymes (APIzym):</i>																
Alkaline phosphatase		60	17	0	29	25	0	33	25	30	50	25	33	67	0	17
Esterase (C4)		90	83	100	88	100	100	83	100	30	100	100	92	67	33	100
Esterase/lipase		100	100	100	100	100	100	83	100	100	100	75	100	67	67	100
Leucine arylamidase		100	100	100	100	100	100	83	75	100	75	25	83	67	0	100
Valine arylamidase		0	0	0	0	0	0	17	0	0	0	0	0	0	0	17
Cysteine arylamidase		60	0	50	23	0	0	17	0	30	80	0	25	0	0	0
Chymotrypsin		90	17	0	100	0	0	0	0	0	0	0	17	0	0	0
Acid phosphatase		90	0	25	100	100	0	83	25	70	100	50	92	100	0	0
Phosphoamidase		100	67	100	100	25	25	83	75	100	100	25	92	33	0	67
α -Galactosidase		0	0	0	65	0	0	0	0	0	0	0	0	0	0	0
β -Galactosidase		90	50	75	100	25	0	67	0	30	0	0	8	0	33	50
β -Glucuronidase		0	0	0	88	0	0	0	0	0	0	0	0	0	0	0
α -Glucosidase		100	0	25	0	100	100	67	25	70	100	25	83	33	0	0
β -Glucosidase		100	100	50	100	50	100	50	0	100	100	25	92	0	0	17
<i>N</i> -Acetyl- β -glucosaminidase		30	0	0	100	0	0	0	0	0	0	0	0	0	0	0
α -Fucosidase		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acid from:</i>																
<i>N</i> -Acetylglucosamine		100	100	100	100	100	100	100	100	100	100	100	100	100	0	100
Adonitol		0	17	100	0	0	0	0	0	0	0	0	17	0	0	0
Amygdalin		100	100	100	100	75	100	67	75	100	50	75	67	33	0	0
Amylose		0	0	0	12	0	25	0	0	0	0	0	0	0	0	0
D(-)-Arabinose		40	0	100	6	0	0	0	0	0	0	25	0	33	0	0
L(+)-Arabinose		30	67	100	100	25	0	0	0	0	25	25	17	33	0	0
Arbutin		10	100	100	100	75	100	100	75	100	75	100	92	67	33	0
D(+)-Cellobiose		100	100	100	100	100	100	100	75	100	100	100	92	100	0	33
Dextrin		90	50	75	100	75	25	67	100	100	50	100	100	100	17	100
Dulcitol		0	17	0	6	0	25	17	0	0	0	25	0	0	0	0
Erythritol		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D(-)-Fructose		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Galactose		100	100	100	100	100	100	83	100	100	100	100	100	100	33	100
Gluconate		70	67	75	100	0	25	17	50	0	25	75	50	33	0	17
Glycerol		100	100	100	100	25	0	0	25	30	50	100	0	33	33	0
Glycogen		10	0	0	6	75	0	0	0	30	0	0	8	0	0	0
<i>meso</i> -Inositol		70	0	0	12	0	25	17	0	33	0	25	0	0	0	0
Inulin		10	17	25	77	50	25	50	0	100	75	50	17	33	0	17
Lactose		100	100	100	100	100	0	0	100	100	100	75	75	67	100	67
Maltose		100	100	100	100	100	100	100	100	100	100	100	100	100	50	100
Mannitol		100	83	100	100	50	50	33	100	100	100	100	17	33	17	17
D(+)-Mannose		100	100	100	100	100	100	100	100	100	100	100	100	100	83	100
D(+)-Melezitose		80	0	100	59	0	50	33	100	30	75	75	0	0	67	50
D(+)-Melibiose		0	50	25	100	100	0	50	50	100	75	75	17	33	33	67
Methyl-D-glucoside		20	0	100	100	25	0	17	75	100	100	0	50	33	0	50
Methyl-D-mannoside		20	0	50	23	0	0	0	100	70	25	0	0	67	0	17
Methyl-D-xyloside		0	0	0	6	0	0	0	0	0	0	25	8	0	0	0
Mucate		10	0	0	12	25	0	0	0	0	0	0	0	0	0	0
D(+)-Raffinose		0	0	25	100	100	25	83	50	100	75	75	8	67	17	67
Rhamnose		50	17	100	12	0	50	17	0	0	25	50	0	0	0	17
Ribose		100	100	100	100	100	100	67	25	100	25	100	100	33	67	50

Table 3 (*continued*)

Test	Phenon ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Salicin		100	100	100	100	100	100	100	50	100	100	100	100	67	0	67
Sorbitol		100	83	100	100	0	0	17	100	100	100	75	17	0	17	17
L(-)-Sorbitose		10	0	100	0	0	0	0	0	0	0	0	8	0	17	0
Starch		100	67	25	100	100	0	33	100	70	75	50	100	100	17	50
Sucrose		80	67	50	100	100	50	100	100	100	100	100	25	67	100	83
D(-)-Trehalose		100	83	100	100	25	25	100	100	100	100	100	100	100	33	17
D(+)-Xylose		40	0	100	100	0	0	0	0	70	75	50	42	67	17	0
L(-)-Xylose		10	0	100	18	0	0	0	0	0	0	0	0	0	0	0
<i>Sensitivity to :</i>																
Ampicillin		100	100	100	100	100	100	100	100	100	100	100	92	100	100	100
Carbenicillin		100	100	100	87	100	75	100	100	100	100	100	100	100	100	100
Furazolidone		90	100	100	100	100	100	100	100	100	100	100	92	100	100	100
Nalidixic acid		0	0	0	23	0	0	0	0	0	0	0	17	0	33	0
Penicillin G		100	100	100	47	100	50	100	100	100	100	100	92	100	100	100
Sulphafuroxazole		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetracycline		100	100	100	82	75	75	100	100	100	100	100	100	100	100	83
	Phenon ...	16	17	18	19	20	21	22	23	24	25	26	27	28		
<i>Colonial characters :</i>																
Colony diameter < 0.2 mm		33	38	100	29	0	0	0	0	10	0	100	0	20		
Colony diameter 0.2-0.4 mm		67	61	0	71	20	100	100	75	60	100	0	100	20		
Colony diameter > 0.4 mm		0	0	0	0	80	0	0	25	30	0	0	0	60		
α -Haemolysis		100	100	33	100	20	0	0	12	60	100	100	0	40		
β -Haemolysis		0	0	33	0	80	100	100	88	30	0	0	0	0		
CAMP test		0	0	0	14	100	0	0	12	50	0	0	0	0		
Red colonies on MacConkey agar		0	0	0	0	80	50	0	0	30	0	0	0	100		
Yellow colonies on TCBS agar		0	0	0	0	0	0	0	0	0	0	0	0	60		
<i>Growth characters :</i>																
At 4 °C		33	0	0	14	0	0	0	0	0	0	0	0	0		
At 10 °C		0	15	0	43	0	0	0	38	0	100	0	67	60		
At 25 °C		100	23	100	100	100	100	100	100	88	100	100	67	100		
At 45 °C		100	38	33	0	20	0	0	0	70	0	0	33	80		
At pH 9.6		0	0	0	0	0	0	0	0	0	0	0	0	20		
With 3% (w/v) NaCl		100	0	67	29	100	100	100	88	100	100	100	33	100		
With 4% (w/v) NaCl		0	0	67	0	100	0	0	62	100	100	67	33	80		
With 6.5% (w/v) NaCl		0	0	0	0	0	0	0	0	0	0	0	0	60		
With 10% bile		67	15	0	100	100	0	50	75	70	100	0	67	20		
With 40% bile		67	0	0	43	0	0	0	0	50	67	0	0	0		
On acetic acid-acetate agar		0	0	0	100	0	0	0	0	0	0	0	0	80		
With sodium azide		0	54	100	14	100	100	100	75	40	67	100	67	60		
With 0.0002% (w/v) crystal violet		33	100	100	57	100	100	0	0	10	100	100	33	100		
With 0.0004% (w/v) crystal violet		0	100	33	100	100	100	0	0	0	100	33	100	80		
On MacConkey agar		0	0	0	0	100	50	0	0	40	0	0	0	100		
On TCBS agar		0	0	0	0	20	0	100	0	0	0	0	0	60		
With thallos acetate		100	77	100	57	100	100	100	100	100	100	100	100	100		
<i>Survival at 60 °C for :</i>																
1 min		100	85	100	100	100	100	100	100	100	100	100	100	100		
15 min		67	15	33	33	100	0	0	88	100	100	100	67	100		
1 h		33	0	33	33	0	0	0	25	100	33	0	0	80		
2 h		0	0	0	0	0	0	0	0	20	0	0	0	0		
<i>Biochemical characters :</i>																
Methyl red		100	92	100	100	100	100	50	88	100	100	100	100	100		
Methyl red (API)		33	38	0	0	40	33	100	88	90	67	100	33	20		
VP		33	38	100	14	20	0	0	12	10	100	0	0	80		
Urease		0	15	0	0	0	0	0	12	0	0	0	0	0		
ONPG		0	0	0	14	0	17	0	50	60	0	33	0	60		
DNAase (plate)		0	0	0	0	40	100	0	25	50	0	67	33	0		
DNAase (API)		0	0	0	0	0	67	0	12	10	0	0	67	0		
Lipase		0	61	67	57	20	0	0	25	60	100	100	67	60		
Phosphatase (plate)		0	23	0	14	100	0	0	100	100	67	0	0	40		

Table 3 (continued)

Test	Phenon ...	16	17	18	19	20	21	22	23	24	25	26	27	28
Aesculin hydrolysis (plate)		0	8	33	43	0	50	0	12	20	100	33	100	80
Aesculin hydrolysis (API)		67	15	0	14	0	33	100	12	10	100	33	100	60
Arginine hydrolysis		100	31	100	0	100	100	100	100	100	100	100	100	80
Casein hydrolysis		0	0	100	0	0	33	0	0	10	0	0	0	0
Gelatin liquefaction		0	0	0	100	0	0	0	0	0	0	0	0	0
Hippurate hydrolysis		0	8	0	14	100	17	0	12	100	100	100	67	40
Starch hydrolysis		0	46	0	14	0	0	50	62	80	100	100	100	80
H ₂ O ₂ production		67	100	67	0	0	0	0	12	0	67	67	67	40
H ₂ S production		0	0	0	0	0	0	0	0	0	67	0	0	0
Dextran production		100	31	0	0	0	17	0	0	10	0	0	0	60
Levan production		0	0	0	14	0	17	0	0	70	0	0	0	20
Arginine decarboxylase		100	100	33	43	80	100	100	62	70	67	100	67	100
Lysine decarboxylase		0	85	33	14	0	0	0	0	0	0	0	33	0
Ornithine decarboxylase		33	77	33	14	20	33	0	0	10	0	0	67	0
Reduction of:														
Janus green		0	0	0	14	0	0	0	100	80	100	100	33	80
Litmus		100	100	67	100	40	17	50	75	90	100	100	67	100
Methylene blue		0	8	67	100	0	0	50	12	70	0	0	67	40
Nitrate		0	8	67	29	0	0	100	25	0	0	0	0	0
Nitrite		67	92	67	100	0	83	100	75	50	0	67	67	60
Selenite		33	69	33	86	80	100	0	75	100	0	100	0	40
Tellurite		0	0	0	0	0	0	100	85	60	0	0	33	0
Tetrazionate		33	92	100	57	80	100	100	100	0	67	100	100	40
Tetrazolium		0	100	67	100	100	100	0	100	80	100	67	100	100
Final pH <4.25		67	92	100	14	100	0	50	50	70	0	33	0	60
Final pH 4.25-4.75		33	8	0	0	0	100	50	50	30	100	67	100	40
Final pH >4.75		0	0	0	86	0	0	0	0	0	0	0	0	0
Clot in litmus milk		0	15	0	0	40	17	50	38	60	0	0	0	80
Oxidation of ethanol		67	54	0	14	100	33	0	25	0	0	0	33	20
Citrate utilization		0	0	0	0	0	0	0	0	0	0	0	0	0
Malonate utilization		0	0	0	0	0	0	0	0	0	0	0	0	0
Enzymes (APIzym):														
Alkaline phosphatase		0	31	67	29	100	100	100	88	100	100	100	0	40
Esterase (C4)		67	77	100	86	83	83	100	88	90	67	100	100	40
Esterase/lipase		100	100	100	100	83	83	100	100	100	100	100	100	80
Leucine arylamidase		67	61	100	0	100	100	100	100	80	100	100	100	60
Valine arylamidase		0	8	0	0	33	33	0	25	0	33	0	0	20
Cysteine arylamidase		0	0	67	0	67	67	0	62	90	0	0	0	0
Chymotrypsin		0	8	33	0	0	0	0	25	0	33	0	67	0
Acid phosphatase		33	46	100	14	100	100	100	100	100	100	100	0	40
Phosphoamidase		33	54	100	0	0	100	100	100	100	100	33	0	20
α-Galactosidase		0	0	0	29	0	0	0	0	0	0	0	0	0
β-Galactosidase		0	15	67	14	0	0	0	25	0	0	0	67	0
β-Glucuronidase		0	0	0	0	17	17	100	88	90	33	100	33	0
α-Glucosidase		33	38	100	29	0	100	50	100	100	67	100	67	20
β-Glucosidase		0	0	33	0	0	0	0	12	10	0	0	33	20
N-Acetyl-β-glucosaminidase		0	8	0	0	0	0	0	12	0	0	0	67	20
α-Fucosidase		0	0	0	0	100	0	0	0	0	0	0	0	0
Acid from:														
N-Acetylglucosamine		100	92	100	86	100	100	100	100	100	100	100	100	100
Adonitol		0	0	0	0	0	0	0	0	0	0	0	0	0
Amygdalin		67	15	0	14	20	0	0	12	20	100	67	33	60
Amylose		0	0	0	0	0	0	0	12	10	0	0	0	20
D(-)-Arabinose		0	8	0	0	20	0	0	0	50	0	0	0	20
L(+)-Arabinose		33	0	0	71	0	0	0	0	30	0	33	0	60
Arbutin		33	38	33	14	20	0	0	25	60	100	67	67	40
D(+)-Cellobiose		67	31	33	14	20	83	100	62	30	100	100	100	80
Dextrin		67	77	0	57	40	100	0	100	100	100	100	100	20
Dulcitol		0	0	0	0	0	0	0	0	10	33	33	0	0
Erythritol		67	0	0	0	0	0	0	0	30	0	0	0	20
D(-)-Fructose		100	100	100	86	100	100	100	100	100	100	100	100	100

Table 3 (*continued*)

Test	Phenon ...	16	17	18	19	20	21	22	23	24	25	26	27	28
Galactose		100	100	100	86	100	100	100	100	100	100	100	100	100
Glucanate		0	15	0	71	0	0	0	0	60	33	67	0	60
Glycerol		100	54	0	0	20	17	50	88	30	100	100	0	100
Glycogen		33	23	0	0	0	50	100	88	10	33	100	100	0
<i>meso</i> -Inositol		0	31	0	0	0	0	0	0	20	33	0	0	40
Inulin		100	31	0	14	0	0	0	12	10	100	0	100	0
Lactose		100	100	100	43	100	100	0	62	60	100	100	100	80
Maltose		100	100	100	100	100	100	100	100	100	100	100	100	100
Mannitol		0	38	0	57	0	33	0	0	30	100	100	33	20
D(+)-Mannose		100	100	100	86	100	100	100	100	100	100	100	100	100
D(+)-Melezitose		0	69	0	14	0	17	0	38	50	0	67	33	60
D(+)-Melibiose		0	77	33	57	20	17	0	12	30	33	100	67	40
Methyl-D-glucoside		33	38	0	71	0	50	100	88	70	33	100	100	40
Methyl-D-mannoside		0	15	0	14	0	0	0	0	10	33	67	33	20
Methyl-D-xyloside		0	0	0	0	20	0	0	0	0	0	0	0	0
Mucate		0	0	0	0	0	0	0	0	0	0	33	0	0
D(+)-Raffinose		0	61	0	43	20	17	50	0	0	33	67	100	40
Rhamnose		0	8	0	0	0	0	0	0	10	0	67	0	60
Ribose		67	92	100	100	100	0	100	100	90	100	100	100	100
Salicin		67	54	67	14	80	100	100	88	100	100	100	100	60
Sorbitol		0	23	0	0	0	33	0	38	20	100	100	67	0
L(-)-Sorbitose		33	0	0	0	20	0	0	0	0	0	0	0	0
Starch		33	31	0	29	20	83	100	100	80	33	100	100	40
Sucrose		100	100	100	100	100	100	100	100	100	100	100	100	60
D(-)-Trehalose		100	77	100	71	100	100	0	75	100	100	100	100	60
D(+)-Xylose		0	0	0	14	0	0	0	12	20	33	0	0	20
L(-)-Xylose		0	0	0	0	0	17	0	0	20	0	0	0	0
<i>Sensitivity to:</i>														
Ampicillin		100	92	100	100	100	100	100	100	100	100	100	67	100
Carbenicillin		100	100	100	100	100	100	100	100	100	100	100	100	100
Furazolidone		100	100	100	100	100	100	100	100	100	33	100	100	20
Nalidixic acid		0	0	0	0	0	0	50	0	0	0	0	0	0
Penicillin G		100	100	100	100	80	83	100	100	100	100	100	100	100
Sulphafuroxazole		0	0	0	0	0	0	0	12	0	0	0	0	0
Tetracycline		100	100	100	100	100	100	100	100	100	100	100	100	80

The lower part of Fig. 1 contains three remaining main areas. The first are classic pyogenic streptococci, referred to as the pyogenic species group. The second contains species that have some of the clinical attributes of pyogenic organisms: this is named the parapyogenic species group. Lastly, there is a well separated though somewhat heterogeneous group of pediococci.

Description of phenons

The phenons are described in relation to Fig. 1. The identity of the ungrouped strains in that figure can be determined by consulting Table 1, where they are given in the same order as in the phenogram. The parameters of the phenons, with intercentroid distances, are shown in Table 2, and Table 3 gives the percentages of positive test reactions.

Enterococcal species group (phenons 1 to 4). These organisms are able to grow at pH 9.6, at both 10 °C and 45 °C, and with 6.5% NaCl and 40% bile. They grow on MacConkey agar and usually give yellow colonies on TCBS agar. These features are characteristic, though they are occasionally found in other groups, particularly pediococci. The strains react with Lancefield Group D antisera.

Phenon 1 is *S. faecalis*. It is distinguished in this species group by hydrolysing hippurate and arginine, by reducing tellurite, and by seldom growing at 4 °C. Strain PB129 (received as *S. faecium*) may have become mislabelled.

Phenon 2 contained strains received as *S. faecium* and '*S. durans*'. Although the three strains with the latter name grouped together within phenon 2, their separation was too small to justify a

separate phenon. Phenon 2 is characterized by ability to grow at 4 °C, and is hippurate negative but arginine positive.

Succeeding phenon 2 in Fig. 1 are three ungrouped strains. PB16 was received as '*S. avium*', and contains the Q antigen, though it links to the base of phenons 1 and 2. Strains PB125 and PB140 likewise seem to be aberrant strains of a similar nature.

Phenon 3 is '*S. avium*', of Nowlan & Deibel (1967), which has the Q antigen as well as reacting with D antiserum. It is unusual among streptococci in giving acid from adonitol, D-arabinose, L-sorbose and L-xylose. It shares with phenon 4 the unusual ability to produce acid from L-arabinose and D-xylose, and also (with some strains of *S. faecium*) to grow on acetic acid-acetate agar.

Following phenon 3 is an ungrouped strain of '*S. faecalis* subsp. *malodoratus*'.

Phenon 4 comprises 17 strains received from Dr E. M. Barnes. They were isolated from the bowel of young chickens (Barnes *et al.*, 1978) and were noted to be unusual in growing slowly on thallos acetate tetrazolium agar (Barnes, 1956) at room temperature, on which they gave pink or red colonies. They reacted with Lancefield Group D antiserum. These strains form a tight cluster at 90% *S* and are one of the most compact of the phenons ($\bar{d}_j = 0.213$, see Table 2). The phenon is referred to as the Chicken Group D.

These strains are very active metabolically (mean vigour = 0.581). They are unusual among streptococci in being positive for *N*-acetylglucosaminase. They resemble phenon 1 in being positive to chymotrypsin, and phenon 2 in being ONPG positive. They are most similar to phenon 3, but differ in being adonitol and L(-)-sorbose negative but raffinose positive.

Following phenon 4 are nine ungrouped strains. The first (PB53) was received as a motile streptococcus similar to *S. faecium*, as was PB153 a little lower down. Two strains (PB154, PB143) are of serological Group K and one (PB156) is one of Group L; these have unexpectedly joined the base of the enterococcal grouping instead of the paravidans grouping. PB109 and PB110 are '*Aerococcus catalasicus*' of Clausen (1964) and PB169 is a pediococcus: these three may be misplaced from phenons 13 or 28.

Paravidans species group (phenons 5 to 11). This area contains clear-cut clusters with no ungrouped strains. These phenons are predominantly aesculin positive (like several other species groups), but they are hippurate negative and seldom reduce the pH of glucose broth below 4.75.

Phenon 5 is clearly *S. bovis*, though the type strain was not included. It shares many features with phenon 6.

Phenon 6 is *S. equinus*. Like phenon 5 it does not hydrolyse arginine or reduce selenite, does not grow with 40% bile, and does not acidify sorbitol or methyl-D-mannoside. Phenon 6, however, does not acidify lactose, melibiose or starch, but commonly acidifies melezitose (unlike phenon 5).

Phenon 7 is *S. salivarius*. The type strain is serological Group K, as are several other strains in the phenon. The two strains named '*S. hominis*' did not form a separate subgroup. The MG streptococcus is also in phenon 7, together with three strains from Professor J. Carlsson. Two of the latter were in the same cluster as *S. salivarius* in his study (Carlsson, 1968). Phenon 7 is lactose negative like phenon 6, but reduces selenite (unlike phenons 5 and 6).

Phenon 8 may be heterogeneous, though it is referred to here as '*S. casseliflavus*' because it contains two motile strains of that name from vegetation (Mundt & Graham, 1968). The other two strains, received as '*S. mutans* subsp. *sobrinus*' and *S. equinus*, may be misplaced from nearby groups. Phenon 8 is characterized by acidifying methyl-D-mannoside and by being β -glucosidase negative.

Phenon 9 is a small phenon containing one strain of *S. bovis* (possibly misplaced from phenon 6) and the type strains of *S. rattus* and *S. mutans*. The last two have a similarity of 86%, but on the present evidence it seems best to treat this phenon as one species, *S. mutans*. Phenon 9 gives small non-haemolytic colonies, and acidifies inulin.

Phenon 10 contains two Lancefield Group N strains of *S. raffinolactis* (Orla-Jensen & Hansen, 1932) from raw milk and two others of unknown serological group from raw carrot (Garvie *et al.*,

1981). They reduce selenite but not nitrite, and are non-haemolytic. They give negative VP and lipase reactions. Three were raffinose positive.

Phenon 11 is a small group of strains from the human mouth referred to as Oral Group I. They are fastidious, being inhibited by 0.1% sodium azide, 10% bile and 3% NaCl, and they grow poorly at 25 °C or less. They are notably sensitive to heating at 60 °C (though the accuracy of the method for the short time period of 1 min is admittedly not very great). They do not reduce tetrathionate, but acidify glycerol.

Lactic species group (phenon 12). This contains strains of *S. lactis*, *S. cremoris*, '*S. lactis* subsp. *diacetylactis*' and '*S. lactis* subsp. *alactosus*'. The full phenogram shows no evidence of internal groupings corresponding to these names. The phenon, we believe, represents one species, *S. lactis*. It is difficult to characterize by single tests, but most strains grow at 10 °C but not at 45 °C, give positive results with the methyl red test, are acid phosphatase positive, and decarboxylate arginine.

Aerococcal group (phenon 13). This is a loose group containing two unidentified streptococci and one strain received as *Aerococcus viridans* (but not the type strain). It is referred to as the aerococcal group. These strains grow both at 10 °C and 45 °C, but not with 40% bile; they hydrolyse starch and are sensitive to 0.1% sodium azide.

Phenon 13 is followed by an ungrouped strain PB201, which resembles *S. salivarius* in many ways.

Thermophilic species group (phenon 14). The six strains of *S. thermophilus* comprise this well-defined phenon. They grow well at 45 °C and with 40% bile, but not at 10 °C nor on MacConkey agar [Deibel & Seeley (1974) report no growth with 40% bile]. They do not acidify cellobiose, galactose, salicin and *N*-acetylglucosamine. They lack leucine arylamidase.

Four ungrouped strains follow phenon 14 and are attached near its base (see Discussion).

Viridans species group (phenons 15–18). These phenons are usually α -haemolytic and commonly produce H₂O₂. They tend to be negative for aesculin hydrolysis, acid phosphatase, janus green reduction and D-xylose fermentation. They seldom grow with 4% NaCl, but often grow at 45 °C. Three ungrouped strains occur above phenon 15 in Fig. 1. They are unidentified streptococci, PB43, PB170 and PB46.

Phenon 15 is presumably *S. mitis*, though the type strain was not studied. Two strains are Lancefield Group O. These strains do not reduce nitrate, but they acidify dextrin.

Phenon 16 is *S. sanguis*. It has several distinctive properties: it produces dextran and acidifies inulin and glycerol. It is unusual among streptococci in commonly acidifying erythritol and oxidizing ethanol.

Following phenon 16 are six ungrouped strains, described in more detail in the discussion: the first four link near phenon 16 and the last two near phenon 17.

Phenon 17 is referred to as Oral Group II, and forms at 84% similarity. The strains mainly belong to clusters I and V of the numerical taxonomy of Carlsson (1968). They are strongly α -haemolytic, reduce tetrazolium, grow well with 0.0004% crystal violet, but are inhibited by 3% NaCl. Few grow at 25 °C. About half oxidize ethanol. They are unusual for streptococci in being commonly positive for lysine decarboxylase. They differ from phenon 16 in being usually aesculin negative and raffinose positive.

Phenon 18 is '*S. milleri*' of Guthof (1956). The three strains group tightly, and they are distinctive in giving small colonies, growing well with sodium azide, in producing no dextran and not acidifying dextrin. They are usually cysteine arylamidase positive and reduce nitrate. They are unusual among streptococci in hydrolysing casein.

Three ungrouped strains follow phenon 18. The second, PB70, is the only strain of *S. pneumoniae* that survived all examinations.

Pneumococcal species group. This was represented only by PB70, as just noted. The strain was soluble in bile, as were only two other strains in the survey, PB145 and PB102, both ungrouped after phenon 16.

Leuconostoc group. This contains phenon 19 containing six strains of *Leuconostoc* and one of the sole species of *Gemella*, *G. haemolysans*. Though heterogeneous, it is reasonably well separated from other phenons. It is characterized by α -haemolysis, gelatin liquefaction, good growth with 0.0004% crystal violet and on acetic acid-acetate agar, sensitivity to 4% NaCl, and lack of leucine arylamidase. The final pH in glucose broth is usually over 4.75.

Near the base of phenon 19 is an ungrouped strain (PB168) of *Pediococcus halophilus*.

Pyogenic species group (phenons 20–24). This group comprises mainly β -haemolytic strains. They usually reduce tetrathionate, and hydrolyse arginine, but seldom aesculin. They seldom acidify sorbitol. They usually give a positive reaction in the plate method for phosphatase, and in the APIzym kits they commonly show reactions for acid phosphatase, phosphoamidase, cysteine arylamidase and β -glucuronidase.

Phenon 20 is classical *S. agalactiae* from bovine mastitis. All the strains are believed to come from bovine sources. The phenon is characteristically CAMP positive, usually β -haemolytic and gives a final pH below 4.25 in glucose broth. It grows with 4% NaCl, hydrolyses hippurate, and is unusual among streptococci in being α -fucosidase positive and oxidizing ethanol.

Phenon 21 is *S. pyogenes*. It is a compact group of β -haemolytic strains, distinctive in giving positive DNAase reactions. They do not grow with 4% NaCl nor 10% bile, and do not ferment ribose.

Phenon 22 contains only two strains of *S. equi* which derive from the same isolate. It grows on TCBS agar, reduces nitrate and tellurite, is lactose, dextrin and trehalose negative, and showed no cysteine arylamidase.

Phenon 23 contains eight strains. Four were received as '*S. equisimilis*', and two as '*S. zooepidemicus*'. These were all serological group C. Two rather peripheral strains were an unidentified group G streptococcus (PB44), and an unidentified group L streptococcus (PB155). The last two may have been misplaced, and should perhaps have been excluded. The phenon was named '*S. equisimilis*'. Distinctive features are ability to reduce janus green and usually tellurite and selenite, and inhibition by 0.0002% crystal violet.

Two ungrouped strains (PB45, PB142) join near the base of phenon 23.

Phenon 24 was composed of human isolates of serological group B; the last six (PB134 to PB136 in Table 1) were more tightly grouped than the rest. The group is referred to as Clinical Group B. Most strains were CAMP positive, but unlike phenon 20, none were positive for α -fucosidase: they differed from phenon 20 also in being more sensitive to crystal violet and in being more active in reducing methylene blue, janus green and tellurite, and none oxidized ethanol nor reduced tetrathionate. The phenon is one of the least compact ($\bar{d}_J = 0.31$).

Parapyogenic species group (phenons 25–27). This species group is similar to the last, but strains are aesculin positive, usually acidify amygdalin, sorbitol and mannitol, are cysteine arylamidase negative, and less often positive in the plate method for phosphatase.

Three ungrouped strains occur before phenon 25. Two (PB42, PB122) are of serological group E, the other (PB191) was of group M.

Phenon 25 is *S. uberis*, a distinctive species which is α -haemolytic, VP positive and produces acid from glycerol and inulin. It possesses acid and alkaline phosphatases and does not reduce selenite. It is unusual for streptococci in often giving H_2S from thiosulphate. Deibel & Seeley (1974) report that it grows at 45 °C, but our strains did not.

Phenon 26 is '*S. dysgalactiae*': it is α -haemolytic, but the colonies are smaller than those of *S. uberis*, from which it also differs in being VP negative, inulin negative and in reducing selenite.

An ungrouped strain of serological group P follows phenon 26.

Phenon 27 contains one strain each of serological groups R, S and T, and is named therefrom. The strains were not haemolytic. They were VP negative and glycerol negative, but inulin positive. They lacked acid and alkaline phosphatases and were negative in the plate test for phosphatase. They did not reduce selenite. They were unusual among streptococci in commonly possessing ornithine decarboxylase and *N*-acetylglucosaminase.

Pediococcal group (phenon 28). The final phenon represents most of the genus *Pediococcus*.

Also included was another strain received as *Aerococcus viridans*. The phenon is heterogeneous, forming at 73% similarity, but is reasonably well separated from the others. It has few distinctive properties. It resembles the enterococcal species group in having wide tolerance to temperature, salt and pH and in giving red colonies on MacConkey agar. It differs from that group in being inhibited by 40% bile and by not acidifying sorbitol. Arginine, aesculin and hippurate are commonly hydrolysed.

Distinctness of phenons and their relationships in phenetic space

Overlap of phenons. None of the possible pairs of the 28 phenons overlapped by more than 1%: the great majority showed overlap of less than 10^{-6} . When tested against the null hypothesis of a rectangular distribution, there were 45 pairs where the number of strains were too few to permit a significance test. Most of the overlaps could be shown to be significantly less than the null hypothesis at better than the 0.9 confidence level, but four did not quite reach 0.9 confidence. They, with the overlap in parentheses, were the phenon pairs 7 and 11 (0.91%), 7 and 12 (0.91%), 8 and 15 (0.17%) and 24 and 28 (0.15%). One additional statistical point was briefly investigated. The *W* statistic (Sneath, 1977), implies continuous, not binary, variables, which must reduce the degrees of freedom. Thus, if only 20 characters vary out of 100, and give a mean similarity of 90% between clusters, the effective degrees of freedom must be nearer 20 than 100. One possible estimate of n' , the effective number of variable characters, is $2n(1 - \bar{S}_{SM})$ where $\bar{S}_{SM}$ is the mean of the similarities between members of one cluster and members of the other. In this study n' was usually about 60, but the reduction from 136 to this level had little practical effect on the significance tests.

Principal coordinates analysis. Figure 2 gives a general view of relations between the phenons comparable to the phenogram in Fig. 1. It should be noted that ordination techniques do not reflect exactly the same aspects of the multidimensional relationships as phenograms. This is because some phenons have larger loadings than others on the major ordination axes, which emphasizes selectively some of the interphenon relationships. This has happened here to a minor extent: the separation between the phenons of the enterococcus species group (phenons 1–4) is greater than that between the viridans species group (phenons 15–18), although the separation is about the same in the full hyperspace (as shown by Fig. 1). The reason is presumably because the distinction between the enterococci and the rest of the genus is very marked, so that this distinction is strongly expressed in the first three axes and hence in Fig. 2. The apparent overlaps between the closer phenons is, however, exaggerated in Fig. 2 because there are only three dimensions in the figure; in the original hyperspace the overlap is very small.

The variance on the first three principal axes (indicated by the sum of the eigenvalues) is only 38.7% of the total, which is rather low for comparable analyses (e.g. Sneath, 1978; Sneath *et al.*, 1981). Nevertheless, the main taxonomic structure of the genus is well shown. The enterococcal species group (phenons 1–4) is well separated on the right. The paraviridans species group (phenons 5–11) is at the back. The lactic group (phenon 12), the aerococcal group (phenon 13) and the thermophilic group (phenon 14) are spread across the diagram in the middle distance. The viridans species group (phenons 15–18) is on the lower left, with the leuconostocs (phenon 19) nearby. The pyogenic species group (phenons 20–24) is near the front, and the parapyogenic species group (phenons 25–27) is high up in the middle distance. The pediococci (phenon 28) lie behind the pyogenic species.

The most typical species of the genus, in the sense of being closest to the grand centroid of the configuration, are the paraviridans and lactic species, and the most atypical are the enterococci and some of the pyogenic species. There is a trend in vigour values, from low vigour of the phenons on the lower left to high vigour of those on the upper right.

Other findings

Single Linkage clustering with S_G . Single Linkage clustering showed almost the same phenons as UPGMA, but their distinctness was much reduced, and their position in the phenogram often

Fig. 2. Principal coordinates analysis of the distances between centroids of the 28 phenons. The phenon numbering is the same as in Fig. 1. The intercentroid distances and radii of phenons are from Table 2. The former were scaled by $\sqrt{136}$ and the latter were obtained by multiplying d_i by $\sqrt{(3/136)}$, so as to take account of the number of characters and the reduction from 136 to 3 dimensions. The first three principal axes, I, II and III, are shown, and the corresponding eigenvalues are 60.17, 32.55 and 28.08, together comprising 38.66% of the total variation.

differed. The most notable changes were as follows. There was less distinction between the enterococcal and paraviridans species groups, and phenons 3 and 4 moved into the paraviridans area. The pyogenic, parapyogenic and thermophilic species groups were to some degree intermingled. Almost all the aerococci, pediococci and leuconostocs came at the foot of the phenogram.

S_{SM} and Vigour and Pattern Coefficients. The phenograms from *S_{SM}* were almost the same as those from *S_G*. The composition of the phenons was almost identical. There was some movement of the phenons, with pediococci becoming allied to the enterococci, and phenons 25 and 26 joining the paraviridans species group. The two strains of '*S. zooepidemicus*' were closer to *S. equi* than to '*S. equisimilis*'.

The vigour of the phenons does not vary greatly, ranging from 0.34 for *S. thermophilus* to 0.58 for Chicken Group D. In consequence, the results of clustering with the Pattern Coefficient *D_P* are not greatly different from those with the other coefficients. The UPGMA phenogram with *D_P* showed almost identical phenons as that with *S_G*. The pediococci were loosely allied to a region comprising enterococcal, lactic and paraviridans phenons: within this complex *Streptococcus lactis* has moved near *S. faecalis* and *S. faecium*, and '*S. avium*' and Chicken Group D were near the paraviridans phenons. The pyogenic species group showed little change except that '*S. zooepidemicus*' was again closer to *S. equi* than to '*S. equisimilis*'. *Streptococcus sanguis* and *S. mitis* have become loosely linked to the pyogenic species group, and '*S. milleri*' and Oral Group II were next to the leuconostocs. Many of the differences involved small changes in the branching levels, and may be of little significance. The movement of certain single strains is considered in the Discussion.

Cophenetic discrepancies. It has been noted that most of the distortion between a similarity matrix and a phenogram derived from it is often due to a few of the OTUs (Sneath & Sokal, 1973; p. 282). These 'poorly fitting' OTUs are commonly satellites to clusters, or strains showing instability in different cluster analyses. An example was given by Sneath *et al.* (1981). In the present study, the highest distortion Δ_j in the *S_G* UPGMA analysis was usually shown by satellite strains, or those peripheral in their own clusters. There was a tendency for phenons of high vigour to have more strains than the average with high Δ_j , as noted by Sneath *et al.* (1981), but, unlike that study, here there was no tendency for strains of low vigour to have high Δ_j values. We still lack any clear understanding of the biological reason for these findings.

Discriminatory tests. An identification matrix was made by choosing 60 tests of good discriminatory power. This was evaluated to confirm that good-quality matrices could be constructed for streptococci. The matrix showed no pairs of phenons that overlapped more than 0.5%, and the most typical strains possible showed Willcox probability scores (Willcox *et al.*, 1973) of over 0.99999 for every one of the 28 phenons. An analysis (*S_G*, UPGMA) from these 60 tests gave almost exactly the same phenons as Fig. 1, and the only notable difference was movement of phenons 25 and 27 to the paraviridans area. The overlap between the closest phenons was reduced, usually by a factor of 10 or more, and this would be expected because the most discriminatory tests were selected.

For a practical identification system, however, other considerations arise (e.g. reliability and ease of use, diagnostic tests for certain groups). It is therefore hoped to extend this work to give a more practically useful system.

Mole percent GC ratios. The results on 34 strains are shown in the comments section of Table 1. A few phenons are not represented because of technical difficulties in preparing the DNA.

DISCUSSION

Test reproducibility

The difference in test reproducibility for the replicated strains and for those later found to derive from the same isolate is probably due in the main to experimental conditions (though genetic changes may play a part). The replicates were performed under more tightly controlled

conditions than the others. Nevertheless, the strains from the same isolates almost always came in the same phenon.

The results with APIzym kits showed some dependence on the medium on which the strains were grown, as has been noted by others (Odds *et al.*, 1978). The differences from the findings of Humble *et al.* (1977) and Waitkins *et al.* (1980) may well be due to differences in growth medium. Most such differences involved varied grades of reaction, but one or two were clear cut. Thus Waitkins *et al.* (1980) found that *S. faecalis* and *S. bovis* were strongly positive for cysteine arylamidase, whereas we found them negative or weakly reacting. They found *S. faecalis* was negative for β -galactosidase and β -glucosidase, whereas we found it usually strongly positive for both. The kits only detect constitutive enzymes and those induced by the growth medium, and it is therefore evident that careful standardization of the growth medium is important for APIzym tests.

The results in Table 3 are broadly in agreement with those reported by Feltham & Sneath (1982) for a wide range of streptococcal groups. Most differences are probably due to differences in the strain composition of the groups in that study and the present one (the earlier study followed more closely the traditional groupings of streptococci), but other differences are accounted for by technical methods (the previous study emphasized rapid tests with API 50E and API 20E diagnostic kits).

Main structure of the genus

The main areas shown in Fig. 1 largely correspond to traditional sections of the genus (Deibel & Seeley, 1974; Wilson & Miles, 1975; Jones, 1978). The distinctness of the enterococcal species group, and the relative closeness of the pyogenic and viridans streptococci, was noted in early numerical taxonomic work (Raj & Colwell, 1966; Seyfried, 1968; Davis *et al.*, 1969). This is supported by DNA-RNA pairing (Weissman *et al.*, 1966), which is low between pyogenic and viridans streptococci, but very low between these and *S. faecalis*. This gives some support for the proposal (Kalina, 1970) that the enterococci merit a genus of their own. The lactic streptococci may be closer to the enterococci than is indicated by the S_G results, though the D_P results point toward it. *Streptococcus lactis* is closer to *S. faecalis* than are the viridans and pyogenic streptococci as judged by the serological properties of fructose diphosphate aldolase (London & Kline, 1973; London *et al.*, 1975).

It seems useful to distinguish between the paraviridans and viridans species groups. The taxonomy of these streptococci has proved exceptionally difficult (particularly the oral species, which have been reviewed recently by Hardie & Marsh, 1978) so that some provisional framework for them is useful. It may well happen, however, that better arrangements of this area, together with the allied lactic, thermophilic and pneumococcal species groups, may emerge from further work. There also seems merit in distinguishing the parapathogenic species group from the classical pyogenic streptococci, although their separation is less marked, and it is possible that '*S. dysgalactiae*' would be better placed with the pyogenic species as suggested by the findings of Feltham (1979).

The present findings are largely in agreement with the numerical taxonomy of Colman (1968), though he studied principally viridans and non-haemolytic streptococci. Many of the phenons are clearly the same. Strains that were ungrouped in his study are commonly ungrouped in the present work, and usually close to similar phenons in both. Similarly, a number of phenons are equivalent to those in the numerical taxonomy of Drucker & Melville (1971), e.g. *S. faecalis*, *S. sanguis*, and their broad groupings of enterococci, viridans streptococci and pyogenic streptococci are not dissimilar to ours: they also found that the *S. salivarius* and *S. mutans* complexes were somewhat separated from the other viridans streptococci, although the enterococci formed a more central group in their analysis than in ours.

Many of our strains are the same as those used by Feltham (1979) in a numerical taxonomy based mainly on API 20E and API 50E test kits. The Average Linkage phenogram in his paper shows most of the phenons recognized in the present study. Among the viridans-like species he had rather few type strains, so the identity of some of his groups is uncertain; this may account for the lack of separation between paraviridans and viridans species groups in his figure. There

are three differences that may be significant, however. Some strains received as *S. agalactiae* formed a cluster in the viridans area. Strains of *S. lactis* and *S. cremoris* formed separate clusters. '*Streptococcus dysgalactiae*' grouped with '*S. equisimilis*', which was distinct from '*S. zooepidemicus*'. The reason for these differences is not entirely clear, though the small number of tests (60) and the few strains (mostly from culture collections) may explain the instability in clustering.

The distribution of Lancefield group antigens in relation to phenetic groups has been sufficiently discussed in the recent literature (Colman, 1968; Colman & Williams, 1972; Deibel & Seeley, 1974; Jones, 1978). Their occurrence in the phenograms is much as was expected. Some phenons always have a particular antigen (e.g. *S. pyogenes*), other antigens (e.g. D, K, M) occur in widely different phenons. The present study is also broadly concordant with cell-wall composition (Colman & Williams, 1965; 1972). There are numerous patterns of carbohydrates in streptococcal cell walls, but many of these differ only in one or two sugars, and similar patterns are seen in most of the enterococcal groups, in most of the pyogenic groups, and so on. Where comparable, our phenons show good concordance with peptidoglycan types summarized by Deibel & Seeley (1974).

Species groups

The structure within the enterococcal species group is in accord with that found by Jones *et al.* (1972) though phenon 4 was not represented there. *Streptococcus faecalis* is now accepted as quite distinct from *S. faecium*, as shown here. The DNA pairing evidence of Roop *et al.* (1974) that suggests these species are very close is not directly relevant, because their strain labelled *S. faecium* shows a predominance of features characteristic of *S. faecalis* (D. Jones, personal communication). There were no groupings within phenon 1 corresponding to '*S. faecalis* subsp. *liquefaciens*' and '*S. faecalis* subsp. *zymogenes*'; this is in keeping with recent work (Deibel, 1964; Jones *et al.*, 1972): the abilities to liquefy gelatin and haemolyse red cells, on which these subspecies are based, is controlled by plasmids (Jacob *et al.*, 1975; Oliver *et al.*, 1977). Similarly, recent work supports the conspecific status of *S. faecium* and '*S. durans*' (see Jones, 1978). The compact phenon 3, whose strains possess the Q antigen, justifies the species '*S. avium*' of Nowlan & Deibel (1967); this name should be revived. Its presence in the enterococcal species group is supported also by its closeness to *S. faecalis* relative to viridans and pyogenic streptococci, as judged by serology of fructose diphosphate aldolase (London *et al.*, 1975). Phenon 4 (Chicken Group D) is sufficiently well separated from other clusters to be worth recognizing as a separate species and has been proposed as '*Streptococcus gallinarum*' (Bridge & Sneath, 1982) with type strain PB21 = NCTC 11428.

The ungrouped strain PB69 of '*S. faecalis* subsp. *malodoratus*', isolated from cheese, is one of the atypical forms discussed by Jones (1978). These may perhaps constitute a separate species. Colobert & Blondeau (1962), in a numerical taxonomy of enterococci, noted there were a number of small groups that differed markedly from *S. faecalis* and from *S. faecium*. The other ungrouped strains in this species group are varied, being similar to enterococci, viridans streptococci or pediococci. They may represent atypical strains of these that have been misplaced because of their rather low similarity to any phenon.

The streptococci of the paraviridans species group come from a variety of habitats – mouth, lower bowel, plants and dairy products. In the past, some have been included in the enterococcal area (*S. bovis*, *S. equinus*). They seem to be more closely allied to the lactic and thermophilic species groups than to the classical viridans streptococci. Many of them belong to Lancefield groups D, N or K. We had too few strains of *S. bovis* to see whether there were biovars corresponding to differences in capsular slime, growth at 22 °C, α -haemolysis and mannitol fermentation, as suggested by Medrek & Barnes (1962) and Keil & Skadhauge (1973), but *S. bovis* may be more heterogeneous than is commonly thought (Garvie & Bramley, 1979b). The separation of *S. bovis* and *S. equinus* is supported by the overlap statistics (overlap $< 10^{-6}$), though strains were few. *Streptococcus salivarius* (phenon 7) is very similar to the group with the same name in the studies of Colman (1968) and Colman & Williams (1972); the only major difference is that the strains in our hands hydrolysed starch. Phenon 7 differs from the group

named *S. salivarius* by Facklam (1977) in being lactose negative, and often inulin negative and α -haemolytic. The MG streptococcus PB92 was included in phenon 7, though earlier work (Williams, 1956) suggested that it was different from *S. salivarius*. The reason may lie in the fact that a variety of physiological and serological forms of viridans streptococci have been given the name MG streptococcus. Phenon 7 also contained two strains of Carlsson's group III, which in his study clustered with *S. salivarius* NCTC 8618 (Carlsson, 1968), but also one of his group IV strains (PB189).

Phenons 8 and 9 are not very satisfactory clusters. The former contains two strains of '*S. faecium* subsp. *casseliflavus*', yellow-pigmented forms from plants (though under our conditions of testing they did not show appreciable pigment). These strains are reported as motile, but they differ considerably from other motile Group D streptococci (two of which, PB53 and PB153, were among the ungrouped strains below phenon 4). There seem to be two groups of yellow-pigmented motile streptococci from plants on DNA pairing evidence (Vaughan *et al.*, 1979), and other reports suggest there may be yet further distinct groups of plant streptococci (Splittstoesser *et al.*, 1968; Amstein & Hartman, 1973; Mundt, 1975). Roop *et al.* (1974) showed by DNA pairing that plant strains were distantly related to *S. faecalis*, and this distinction is supported by their different isoprenoid quinones (Collins & Jones, 1979). The present study, though limited, suggests that a species '*S. casseliflavus*' may well be justified, but that it needs better definition. These strains had much lower metabolic activity (mean vigour = 0.43) than the enterococci. Phenon 9 represents *S. mutans* as shown by the presence of the type strain. We have not shed much light on the *S. mutans* complex, in which there are several named subspecies that Coykendall (1977) proposed should be raised to full species. The occurrence of *S. rattus* (PB58) in phenon 9 is in accord with the high DNA pairing between it and *S. mutans* (Coykendall, 1974), whereas the type strain of '*S. sobrinus*' (PB59) was less close on DNA evidence, and this strain is in phenon 8. Another strain of '*S. sobrinus*' (PB60) is ungrouped after phenon 18, so the species appears heterogeneous. There seems to be some correspondence, too, with the groupings of Facklam (1974). Our phenon 9 corresponds well to *S. mutans* of Colman & Williams (1972) and Facklam (1977) except that our strains ferment raffinose, and partially to *S. mutans* of Drucker & Melville (1971). However, another strain, '*S. mutans* subsp. *mutans*' (PB151) was ungrouped near phenon 14, and phenon 9 also contains a Group D streptococcus (PB8) probably misplaced from phenon 6.

Phenon 10 (*S. raffinolactis* of Orla-Jensen & Hansen, 1932) has been recently redescribed by Garvie (1978). Though belonging to serological group N, the present study confirms the view of Garvie *et al.* (1981) that this species is distinct from *S. lactis* and *S. cremoris* on physiological and DNA pairing evidence. They also found high DNA pairing between the type strain NCDO 617 and the other three strains in phenon 10. Phenon 11 (Oral Group I) contains three strains from cluster II of the numerical taxonomy of Carlsson (1968), and one (PB174) from his cluster III, but this strain was peripheral, and could be regarded as an ungrouped satellite. This phenon resembles *S. mutans* as described by Facklam (1977) in most respects.

The lactic species group contains only phenon 12, which comprises strains of *S. lactis* and *S. cremoris*. These should probably be one species (Jones, 1978). The evidence includes physiological, DNA pairing and cell-wall data. Similarly, there is no evidence here in favour of subspecies status for the strains with the epithet '*diacetylactis*' and '*alactosus*'. Further work by Garvie *et al.* (1981) supports the union of all these into one species, on DNA pairing and the properties of lactate dehydrogenase. They all have the same major isoprenoid quinones (Collins & Jones, 1979). Seyfried (1968) found substantial differences between *S. lactis*, *S. cremoris* and '*S. diacetylactis*' in a numerical study. There has been much confusion over the identity of many of these strains in the past, and this may provide an explanation. However, the differences between strains received as *S. lactis* and *S. cremoris* found by Feltham (1979) were almost always such that *S. cremoris* strains lacked fermentative powers possessed by *S. lactis* at the chosen time of reading the tests (Feltham & Sneath, 1982): it is therefore possible that the name *S. cremoris* is commonly given to slower growing strains of these streptococci, and this would bear further examination. The average vigour of strains received by us as *S. lactis* and *S. cremoris* was 0.454 and 0.380 respectively.

The thermophilic species group also contains only one phenon, *S. thermophilus* (phenon 14), long recognized as a distinctive species (Jones, 1978). It has lower vigour (mean = 0.34) than any other phenon. The strains grew quite well at 37 °C, so there is no reason to suppose they were tested under unfavourable conditions.

The aerococcal group (phenon 13) is discussed later with other strains that are not streptococci. Several ungrouped strains in this region of Fig. 1 may be aberrant members of nearby phenons. PB124 may be an atypical strain of phenon 12, and PB71 (which is Lancefield Group N) is one of the unclassified strains in the study of lactic streptococci by Garvie *et al.* (1981). PB201 and PB151 are somewhat similar to phenons 7 or 9. PB49 is of serogroup O, and may belong near phenon 15.

The viridans species group is an area where the taxonomy is still very confused. There are probably further species to be described and some problems of nomenclature. Hardie & Marsh (1978) give a table of corresponding species in the works of several authors. The serological groups are varied, with antigens F, G, H and O predominating (Colman & Williams, 1972). Phenon 15 can only be tentatively identified as *Streptococcus mitis* because we did not study the type strain, but its features are similar to *S. mitis* as described by Facklam (1977) and to '*S. mitior*' of Colman & Williams (1972). Phenon 16 (*S. sanguis*) contains Group H strains as expected: it fits fairly well with *S. sanguis* of Drucker & Melville (1971) and Colman & Williams (1972), and with *S. sanguis* I of Facklam (1977).

Oral Group II (phenon 17) seemed to us to be sufficiently distinct from other groups to deserve a species name. It has therefore been proposed as a new species '*Streptococcus oralis*' (Bridge & Sneath, 1982) with type strain PB182 = NCTC 11427. We were unable to identify it with existing species. It has some similarity to '*S. mitior*' of Colman & Williams (1972) and *S. sanguis* biotype II of Facklam (1977). The strains were mostly from clusters I and V of Carlsson (1968). Although in his study some reference strains of *S. sanguis* and *S. mitis* were found in his cluster I, this did not clearly indicate identity with either of those species. Coykendall & Munzenmaier (1978) found moderately high DNA pairing between strains PB183, PB185, PB186 and the atypical *S. sanguis* (PB66) which is ungrouped but nearby: they considered the first three to belong to '*S. mitior*'.

'*Streptococcus milleri*' was a species proposed by Guthof (1956). It seems to be distinctive, and has been implicated in human infections, particularly abscesses of brain, lung and liver (Parker & Ball, 1979). Phenon 18 represents this, and agrees well with the description by Colman & Williams (1972), except that in our hands strains were usually aesculin negative. It is related to the 'minute' streptococci of Lancefield Groups F and G. It seems appropriate to revive this name.

Little can be said about the ungrouped strains in the viridans species group region of Fig. 1. Two strains of Lancefield Group M (PB47, PB157) link at 87% similarity, and could be the nucleus of another phenon. Two strains of '*S. suis*' (PB115, PB123) were later found to derive from strain PM1 of Elliott (1966) and may be better placed near phenon 27. PB66 is a strain of *S. sanguis* that Cole & Kolstad (1974) regarded as atypical. The type strain of *S. pneumoniae* comes in this region. The leuconostocs of phenon 19 are discussed later.

The pyogenic species group contains the classical pyogenic streptococci, but there are unresolved problems in those of serological groups B and C. Phenon 20, *S. agalactiae* from bovine mastitis, is in the present study separate from the human Group B strains of phenon 24 (see below). Phenon 21 (*S. pyogenes*) calls for no special comment, but it is unfortunate that phenon 22 (*S. equi*) contained strains derived from the same isolate; the species is obviously under-represented here. Phenons 22 and 23 are somewhat of a puzzle, although the paucity of strains may be the explanation. In phenon 23 there are two strains that may have been misplaced (PB44 and PB155), but the others are all Group C and represent '*S. equisimilis*' and '*S. zooepidemicus*'. The two strains of the latter joined at 92% similarity, but we were reluctant on the present evidence to regard them as a separate phenon. In other analyses these two species were better separated, as in the study of Feltham (1979). The species *S. equi*, '*S. equisimilis*' and '*S. zooepidemicus*' are clinically different in the animal infections they cause (Bryans & Moore, 1972; Ross, 1972). They would well repay more study.

Phenon 24 (Clinical Group B) comprises human strains, most from parturient women or neonates. The evidence for its distinctness from phenon 20 is still equivocal. A recent study by A. Nakkash in this Department indicates a spectrum of intermediates between the human Group B strains and classical bovine mastitis strains. It is possible that the apparent distinction here (also noted by Feltham, 1979) is spurious. All strains in phenon 20 were old ones from culture collections, and had lower vigour than those of phenon 24 (Table 2). In the D_p analysis the phenons, though still distinct, come next to one another. On the other hand, epidemiological evidence suggests that classical *S. agalactiae* causes a specific bovine infection that can only be contracted from other cows with mastitis, despite some equivocal findings (Wilson & Salt, 1978). Further, strains of phenon 20 were unique in the present study in possessing α -fucosidase, and unusual in oxidizing ethanol.

Of the ungrouped strains in the pyogenic species group it may be noted that PB142 is a 'large colony Group G' strain (traditionally considered a pyogenic streptococcus).

The parapyogenic species group is represented by phenons 25 to 27, but it is possible that the ungrouped strains above the former represent another species, *S. acidominimus*, for although PB42 is not in very good agreement with the description of Deibel & Seeley (1974), and was received as '*S. infrequens*', yet these two species are similar to one another and not unlike *S. uberis* (Jones, 1978). All three are often Lancefield Group E, like two of these strains. Another ungrouped strain (PB158) is serologically Group P; this antigen also occurs in these species and de Moor & Thal (1968) have noted the physiological similarity of strains of Groups E, P and U. *Streptococcus uberis* (phenon 25) is distinctive. Garvie & Bramley (1979a) have shown that strains similar to *S. uberis* form at least two distinct groups: all our strains belong to their Group I. Our results are in good agreement with those of Deibel & Seeley (1974) and Facklam (1977). Phenon 26 (*S. dysgalactiae*) is also in reasonable agreement with the literature, except that our strains were glycerol and mannitol positive. The R, S, T Group (phenon 27) seems a natural cluster. Strains with these antigens have been noted to be similar physiologically, and commonly cause septicaemia in pigs. The relationship to '*S. suis*' isolated by Elliott (1966) from young pigs (represented by PB115 and PB123 below phenon 16) is not clear. Elliott found his strains to react with group D antiserum, and suggested they might be allied to *S. faecalis* or *S. bovis*, but Colman (1968) and Jones (1978) did not consider this likely. In the present study they are not very similar to any phenon.

The strains of genera other than *Streptococcus* are mainly in phenons 13, 19 and 28, with a few ungrouped strains. These comprise strains from various species of *Aerococcus*, *Pediococcus*, *Gemella* and *Leuconostoc*. The present study does not contain adequate representatives of these bacteria, but it may be noted that the distinction of these genera from *Streptococcus* is based largely on traditional characters, especially morphological ones. The phenogram gives the impression that *Leuconostoc* may be embedded in *Streptococcus*, but not much weight should be given to this. *Gemella* (though we did not have the type strain of *G. haemolysans*) seems very similar indeed to *Leuconostoc*. The aerococcal group (phenon 13) is notably diffuse, and should perhaps come near the pediococci of phenon 28. Neither of the strains received as *Aerococcus viridans* is the type strain, and they are very different from each other: the taxonomy of this genus seems still confused.

Other findings

The results with the Pattern Difference show that none of the phenons is composed simply of slow-growing or fast-growing strains of other phenons. A few ungrouped strains may be better understood by considering the D_p analysis. PB16 moved to the periphery of '*S. casseliflavus*' in the D_p phenogram, and PB152 moved from the enterococcal area close to *S. mitis*, which would seem more natural. The aberrant *S. salivarius* PB143 moved to the periphery of Oral Group I. Of the ungrouped strains near phenon 14, PB124 moved close to *S. lactis* and PB71 close to *S. raffinolactis*. PB170 moved into *S. mitis*. Two unexpected movements, however, were of PB77 from phenon 1 into phenon 12, and of PB85 from phenon 1 into phenon 14, and these are unexplained.

The relatively small effect of D_p is accounted for by the small range of mean vigour of the

phenons (0.34 to 0.58). This is less than in some genera, e.g. 0.26 to 0.61 for *Serratia* (Grimont *et al.*, 1977) and 0.11 to 0.72 for *Pseudomonas* (Sneath *et al.*, 1981). It may be that predominantly parasitic genera show a narrow range: thus that for *Haemophilus* was 0.35 to 0.55 (Broom & Sneath, 1981).

The mol% GC ratios ranged from 33.2 to 40.4. This is within the range reported for the genus (Jones, 1978), but our values are on the low side compared with those in the literature (Deibel & Seeley, 1974; Hill, 1966). Comparison of results from individual strains reported by Garvie *et al.* (1981) suggests that our values are about 1% lower than theirs on average, and this may perhaps be due to the effect of undue fragmentation of DNA during preparation (Garvie, 1979), or the minor differences in method described earlier. The value for the pneumococcus (PB70) was notably low, but this strain proved difficult to handle when preparing the DNA.

The GC values were, however, quite consistent. Those for the phenons were within the usual range for a single genus, and where more than one strain of a phenon was studied the values were within the 2–3% range expected for species.

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