Preformed enzyme profiles of reference strains of gram-positive anaerobic cocci

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Summary. The preformed (constitutive) enzyme profiles of 30 type strains and reference strains of gram-positive anaerobic cocci were determined with two commercial systems, RapID ANA and a prototype system from API. Both systems identified *Peptostreptococcus anaerobius*, *Ps. asaccharolyticus*, *Ps. indolicus*, *Ps. magnus* and *Ps. micros* accurately, except for one strain of *Ps. magnus* misidentified as *Ps. micros* by the RapID ANA system. The indole-negative, butyrate-producing cocci (classified at present as *Ps. prevotii* and *Ps. tetradius*) produced several different, unique patterns with the prototype API system, but the results with RapID ANA were often misleading. Eight strains of Hare group cocci produced previously described profiles. Four strains of streptococci produced profiles easily distinguished from those of the gram-positive anaerobic cocci. We conclude that most gram-positive anaerobic cocci can be identified rapidly and reliably to the species level by their preformed enzyme profiles, providing that their underlying classification is sound. Problems were encountered with the butyrate-producing cocci, which appear to be a more heterogeneous group of organisms than is currently acknowledged; further taxonomic studies on these organisms are required.

Introduction

Gram-positive anaerobic cocci (GPAC) cause a wide spectrum of disease (Hare *et al.*, 1952; Hare, 1967; Wren *et al.*, 1977; Bourgault *et al.*, 1980; Gorbach, 1985). However, taxonomic difficulties (Weiss, 1981; Ezaki *et al.*, 1983; Huss *et al.*, 1984) have delayed the development of identification techniques of practical use for the clinical laboratory. Gas-liquid chromatography (GLC) is accepted as the method of choice (Holdeman *et al.*, 1977; Sutter *et al.*, 1986), but it has limited powers of discrimination, and the equipment required is expensive and often not available in routine clinical laboratories.

In an earlier report (Murdoch *et al.*, 1988), 69 strains of GPAC isolated from clinical specimens and identified by conventional tests (Sutter *et al.*, 1986), were re-examined, separately and blindly, with two preformed (constitutive) enzyme systems—RapID ANA (Mercia Diagnostics, Guildford) and a prototype system from API (API Laboratory Products, Basingstoke). We have now tested a collection of type and reference strains,

separately and blindly, together with four strains of micro-aerophilic and anaerobic streptococci.

Materials and methods

Bacterial strains

Thirty strains of GPAC and four strains of streptococci were coded at the London Hospital Medical College and examined blindly at St Bartholomew's Hospital; their identities were revealed when all tests had been completed and are shown in table I.

Initial identification

All organisms were characterised by the method of Sutter *et al.* (1986): Gram-stained films were examined; strains were tested for indole production (Indole Spot disks, Lab M, Bury) after incubation for 48 h on non-selective agar (Brucella Medium Base, Difco) with defibrinated horse blood (Oxoid) 6%; they were tested for volatile fatty acid production by GLC (Holdeman *et al.*, 1977) after incubation for 48 h in Robertson's Cooked Meat broth (Southern Group Laboratories, Hither Green, London); and their antibiotic sensitivities were tested with disks containing metronidazole 5 µg, vancomycin 5 µg and SPS (sodium polyanethol sulphonate) 1 mg (Oxoid).

Table I. Identification of organisms

Reference nos. of strains	Source	Received from	Received as	Identified as
NCTC 11460/GIFU 7882/ ATCC 27337(T)	Unknown	T.E.	<i>Ps. anaerobius</i>	<i>Ps. anaerobius</i>
DSM 20357	Abscess	K.H.S.	<i>Ps. anaerobius</i>	<i>Ps. anaerobius</i>
API A73	Unknown	API	<i>Ps. anaerobius</i>	<i>Ps. anaerobius</i>
GIFU 7877	Vaginal discharge	T.E.	<i>Ps. asaccharolyticus</i>	<i>Ps. asaccharolyticus</i>
A/C 21	Unknown	M.W.D.W.	<i>Ps. asaccharolyticus</i>	<i>Ps. asaccharolyticus</i>
NCTC 11029/ATCC 29202(T)	Sheep rumen	A.B.	<i>Ps. heliotrinreducens</i>	<i>Ps. heliotrinreducens</i>
NCTC 11088/ATCC 29427/ GIFU 7848(T)	Secretion of heifer with summer mastitis	NCTC	<i>Ps. indolicus</i>	<i>Ps. indolicus</i>
API A101	Unknown	API	<i>Ps. indolicus</i>	<i>Ps. indolicus</i>
ATCC 29328	Abdominal wound	ATCC	<i>Ps. magnus</i>	<i>Ps. magnus</i>
A/C 6	Unknown	M.W.D.W.	<i>Ps. magnus</i>	<i>Ps. magnus</i>
A/C 8	Unknown	M.W.D.W.	<i>Ps. magnus</i>	<i>Ps. magnus</i>
API A110	Unknown	API	<i>Ps. magnus</i>	<i>Ps. magnus</i>
DW 1	Unknown	G.L.F.S.	<i>Ps. magnus</i>	<i>Ps. micros</i>
DSM 20468/GIFU 7824/ VPI 5464(T)	Purulent pleurisy	K.H.S./T.E.	<i>Ps. micros</i>	<i>Ps. micros</i>
KP 1	Unknown	G.L.F.S.	<i>Ps. magnus</i>	<i>Ps. micros</i>
DSM 20358	Abscess	K.H.S.	<i>Ps. prevotii</i>	I-B +
A/C 5	Unknown	M.W.D.W.	<i>Ps. prevotii</i>	<i>Ps. asaccharolyticus</i>
AN 8	Unknown	G.L.F.S.	<i>Ps. prevotii</i>	<i>Ps. asaccharolyticus</i>
GIFU 7874	Unknown	T.E.	<i>Ps. prevotii</i>	I-B +
STD 1	Unknown	G.L.F.S.	<i>Ps. prevotii</i>	I-B +
WGH 150	Unknown	G.L.F.S.	<i>Ps. prevotii</i>	I-B +
NCTC 9801	Unknown	NCTC	Hare group I	<i>Ps. magnus</i>
NCTC 9803	Infected vagina in puerperium	NCTC	Hare group III	Hare group III
NCTC 9804	Nose	NCTC	Hare group IV	<i>Ps. magnus</i>
NCTC 9805	Normal gingiva	NCTC	Hare group V	<i>Veillonella parvula</i>
NCTC 9808	Faeces	NCTC	Hare group VIIa	I-B +
NCTC 9811	Normal vagina	NCTC	Hare group IX	<i>Ps. magnus</i>
NCTC 9814	Infected vagina in puerperium	NCTC	Hare group III	Hare group III
NCTC 9820	Normal nose	NCTC	Hare group VIII	I-B +
NCTC 9821	Normal tonsil	NCTC	Hare group IX	<i>Ps. micros</i>
NCDO 2227/ATCC 27335(T)	Unknown	NCDO	<i>S. intermedius</i>	<i>S. intermedius</i>
NCTC 11323/ATCC 27824(T)	Lung abscess	NCTC	<i>S. morbillorum</i>	<i>S. morbillorum</i>
SS 1091	Unknown	L.V.H.	<i>S. morbillorum</i>	<i>S. morbillorum</i>
ACTC 33793/DSM 20469/ VPI 0546/GIFU 7866 (T)	Unknown	ATCC/K.H.S.	<i>S. parvulus</i>	<i>S. parvulus</i>

I-B + = indole-negative butyrate-producing coccus (classified at present as *Ps. prevotii* and *Ps. tetradius*).

(T) = type strain of species.

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API API Laboratories, Basingstoke, Hants.
ATCC American Type Culture Collection, Rockville, USA.
NCDO National Collection of Dairy Organisms, Reading, Berks.
NCTC National Collection of Type Cultures, Colindale, London.

Inoculation of preformed enzyme kits

Growth from a single colony was inoculated on to freshly prepared Columbia Agar (Oxoid) with horse blood 6% supplemented with vitamin K 0.1%; the plates

were then incubated anaerobically for 48 h. The purity of the culture was checked at the same time. Kits were inoculated with a bacterial suspension of density McFarland standard no. 4 (bacterial count c. 10^9 cfu/ml) and incubated aerobically at 37°C for 4 h. Reagents were

then added and reactions interpreted according to the manufacturers' instructions. Tests substrates have been previously described (Murdoch *et al.*, 1988).

The RapID ANA kits generated a six digit code which was interpreted with the RapID ANA Code Compendium. The enzyme profiles produced by the prototype API kits were interpreted by consulting data sheets previously constructed (Murdoch *et al.*, 1988).

Results

Only strains of *Streptococcus morbillorum* and *S. intermedius* grew in an atmosphere of air + CO₂ 5% within 7 days. These strains were resistant to metronidazole, but all others tested, including *S. parvulus*, were sensitive. All organisms tested were sensitive to vancomycin, except for NCTC strain 9805 of Hare group V (identified as *Veillonella parvula*).

The three strains of *Peptostreptococcus anaerobius* examined were all pleomorphic, chain-forming coccobacilli that were sensitive to SPS and produced isocaproic acid. They were correctly identified by both enzyme systems. None of the other organisms tested produced isocaproic acid or was sensitive to SPS.

Seven organisms were received as *Ps. micros* or *Ps. magnus*; all produced acetic acid only on GLC analysis. The organism received as *Ps. micros* and two organisms received as *Ps. magnus* were small, chain-forming cocci and were identified by both test systems as *Ps. micros*. Four organisms received as *Ps. magnus* were medium to large cocci arranged in clusters; RapID ANA misidentified one strain as *Ps. micros* because of a positive prolyl- β -naphthylamide reaction (cupule 3), but otherwise they were correctly identified as *Ps. magnus*.

Of the Hare group organisms tested (Hare, 1967), four produced only acetic acid on GLC analysis. Three of these were large cluster-forming cocci identified as *Ps. magnus* by both test systems: NCTC strain 9801 of Hare group I, NCTC strain 9804 of Hare group IV and NCTC strain 9811 of Hare group IX. NCTC strain 9821, however, which also belongs to Hare group IX, was a small chain-forming coccus, which was identified in both systems as *Ps. micros*.

Ten organisms were received as *Ps. asaccharolyticus*, *Ps. indolicus* or *Ps. prevotii*; all produced butyric acid. Both strains of *Ps. asaccharolyticus* and both strains of *Ps. indolicus* were indole-positive by Indole Spot disks and were correctly identified by both test systems. Two strains received as *Ps. prevotii* were also indole-positive; both systems identified them as *Ps. asaccharolyticus*. The four

remaining strains received as *Ps. prevotii* were indole-negative; with the prototype API system they produced heterogeneous reaction profiles which were, however, distinct from those of other strains. The profiles generated by these four strains with RapID ANA were not unique: one organism was identified as *S. morbillorum*, two as *Ps. tetradius* and one as *Ps. magnus*.

Four Hare group organisms produced butyric acid. NCTC strain 9808 of Hare group VIIa was indole-negative; its enzyme profile was similar to organisms received as *Ps. prevotii*. NCTC strain 9820 of Hare group VIII was also indole-negative; however, its enzyme profile was identical to that of *Ps. asaccharolyticus* except for the negative indole reaction. NCTC strains 9803 and 9814 of Hare group III were indole-positive, but the profiles produced by the prototype API system were completely different from those of *Ps. asaccharolyticus* or *Ps. indolicus*, i.e., sugars were utilised and aminopeptidase activity was almost absent. RapID ANA identified both strains as *Ps. asaccharolyticus*, but the profile codes generated (002254 and 022544) were slightly different from those of strains of *Ps. asaccharolyticus* (typically 012544).

Ps. heliotrinreducens produced trace quantities of acetic acid and butyric acid; the RapID ANA profile code (052401) and the API profile were unique. NCTC strain 9805 (Hare group V) was a small gram-negative coccus that produced propionic acid; it was identified as *V. parvula* by both enzyme systems. The four strains of streptococci produced enzyme profiles easily distinguished from those of other species examined. The code generated by the type strain of *S. parvulus*, which is not included in the RapID ANA Code Compendium, was 612560.

Discussion

Analysis of acid end-products by GLC is acknowledged to be a reliable and important part of the identification of anaerobic cocci (Holdeman *et al.*, 1977; Sutter *et al.*, 1986). In this study, we found complete agreement between the identity of strains indicated by GLC analysis and identification by means of the preformed enzyme profile. There was also 90% agreement between identification based on the enzyme profile and the identity of the organism as sent to us. We conclude that preformed enzyme profiles are a valuable method for the identification of gram-positive anaerobic cocci, especially for laboratories with limited facilities.

At present, GPAC strains that produce isocaproic acid are all described as *Ps. anaerobius*, whereas

Table II. Enzyme patterns of GPAC with prototype API system

Reference nos. of strains	Species	Characteristic reaction in cupule number																																
		1	4	5	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33				
NCTC 11460/ GIFU 7882/ ATCC 27337 (T)	<i>Ps. anaerobius</i>	—	—	+	—	—	—	—	+	—	—	—	+	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—		
API A73	<i>Ps. anaerobius</i>	—	—	+	—	—	—	—	+	+	—	—	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—		
DSM 20357	<i>Ps. anaerobius</i>	—	—	+	—	—	—	—	±	+	—	—	+	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—		
GIFU 7877	<i>Ps. asaccharolyticus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	+	+	—	—	+	—	+	—	±	—		
AN 8	<i>Ps. asaccharolyticus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	+	—	—	±	—	—	+	—	+	—		
A/C 5	<i>Ps. asaccharolyticus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	±	—	—	±	—	+	—	±	—	—		
A/C 21	<i>Ps. asaccharolyticus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	±	—	—	±	—	+	—	+	—	—		
NCTC 9803	Hare group III	—	—	±	—	—	—	—	+	+	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	+	—	—	—	+	—	—		
NCTC 9814	Hare group III	—	—	—	—	—	—	—	+	+	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
ATCC 29427/NCTC 11088/GIFU 7848(T)	<i>Ps. indolicus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	+	—	+	+	—	+	+	—	—	±	—	—	±	—	—		
API A101	<i>Ps. indolicus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	+	—	+	—	—	—	—	±	—	±	+	±	+	—	—		
ATCC 29328	<i>Ps. magnus</i>	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	±	±	+	—	+	+	±	±	+	—		
A/C 6	<i>Ps. magnus</i>	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	—	±	±	—	—	+	+	+	±	±	—		
A/C 8	<i>Ps. magnus</i>	+	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	—	—	±	+	—	±	±	—	—	+	+	+	±	±	—		
API A110	<i>Ps. magnus</i>	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	±	+	—	—	+	+	—	+	±	±	—		
NCTC 9801	<i>Ps. magnus</i>	±	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	+	—	+	±	—	—	+	+	—	+	±	+	—		
(Hare group I)																																		
NCTC 9804	<i>Ps. magnus</i>	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	+	—	+	±	—	—	+	+	+	±	±	+	—		
(Hare group IV)																																		
NCTC 9811	<i>Ps. magnus</i>	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	±	±	—	—	+	+	+	+	±	+	—		
(Hare group IX)																																		
DSM 20468/ VPI 5464/ GIFU 7824(T)	<i>Ps. micros</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—		
DW 1	<i>Ps. micros</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	±	+	+	+	+	+	+	+	—		
KP 1	<i>Ps. micros</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	±	+	+	+	+	±	+	—	—		
NCTC 9821	<i>Ps. micros</i> (Hare group IX)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	±	+	+	+	+	±	+	+	+	+	—		
NCTC 11029/ ATCC 27337(T)	<i>Ps. heliotrin- reducens</i>	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	+	—	+	—	±	+	+	+	+	—		
DSM 20358	I—B+	—	—	±	—	—	—	+	+	±	—	—	±	+	—	—	—	+	—	—	+	—	+	—	+	—	+	+	+	+	—	—		
GIFU 7874	I—B+	+	+	—	+	+	—	+	+	—	—	+	—	—	—	—	+	—	—	+	—	+	—	+	—	+	±	—	+	±	—	—		
STD 1	I—B+	±	—	—	—	+	—	+	+	—	—	+	—	—	—	—	+	—	—	±	+	—	+	—	—	+	±	—	+	+	—	—		
WGH 150	I—B+	+	—	—	—	—	—	±	+	—	—	—	—	—	—	—	—	—	—	+	—	+	+	—	—	+	+	+	+	+	—	—		
NCTC 9808	I—B+	—	—	+	—	—	—	—	+	+	—	—	+	—	—	—	—	+	—	—	±	±	—	—	±	+	—	—	±	—	—	—		
(Hare group VIIa)																																		
NCTC 9820	Hare group VIII	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	—	—	—	—	±	—	+	—	+	—	—	—		
NCTC 9805	<i>V. parvula</i>	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	+	—	—	—	—	—		
(Hare group V)																																		

NCDO 2227/ ATCC 27335/ NCTC 11323/ ATCC 27824(T) SS 1091 ATCC 33793/ DSM 20469/ GIFU 7866/ VPI 0546(T)	<i>S. intermedius</i>	+	+	+	+	+	+	-	+	+	-	+	+	-	-	-	+	+	-	+	∓	+	-	+	-	+	+	±	±	-
	<i>S. morbillorum</i>	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	∓	+	∓	±	-	-	∓	-	-	∓	±	-	-
	<i>S. morbillorum</i>	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	∓	+	∓	+	-	-	∓	-	-	±	+	-	-
	<i>S. parvulus</i>	-	±	±	-	-	∓	-	+	+	-	+	+	-	-	-	-	±	-	±	∓	±	-	±	±	+	+	∓	±	-

The results of reactions in cupules 2, 3, 5 and 6 were always negative and have been omitted.
Some results with certain species varied between different batches of media, notably TYRA (cupule 29) with *Ps. magnus*; the most frequently observed result is given. These variable reactions were not important for correct identification.
+ = strongly positive; ± = weakly positive; ∓ = very weakly positive; - = negative.
I – B + indole-negative butyrate-producing coccus.

GPAC strains that produce only acetic acid are identified as *Ps. magnus* or *Ps. micros* according to the size of the coccus (Sutter *et al.*, 1986). Our results and those of an earlier study (Murdoch *et al.*, 1988) are consistent with that classification. However, enzyme profiles reveal considerably greater heterogeneity in the butyrate-producing cocci, and, in particular, suggest that indole production is not a reliable test for their identification.

Indole-positive, butyrate-producing cocci are classified at present as either *Ps. asaccharolyticus* or *Ps. indolicus* (Ezaki *et al.*, 1983; Sutter *et al.*, 1986), but reliable tests and conditions for their differentiation were not fully described by these authors. Their preformed enzyme profiles are very similar (Murdoch *et al.*, 1988), but *Ps. indolicus* appears to be distinguished by a strongly positive alkaline phosphatase reaction, detectable in both enzyme systems. However, NCTC strains 9803 and 9814 of Hare group III, which would be described by Sutter *et al.* (1986) as *Ps. asaccharolyticus*, produce completely different profiles, almost identical to two 'indole-positive saccharolytic' clinical isolates previously described (Murdoch *et al.*, 1988). It is likely, therefore, that Hare group III is representative of a previously undescribed species of indole-positive butyrate-producing coccus.

It has already been noted that the enzyme profile of NCTC strain 9820 (Hare group VIII) is almost identical with that of four reference strains of *Ps. asaccharolyticus* (table II), differing only by the indole reaction. DNA-DNA hybridisation studies have provided evidence that NCTC strain 9820 is homologous with DSM strain 20364, a reference

strain of *Ps. asaccharolyticus* (Huss *et al.*, 1984). The enzyme profile of NCTC strain 9820 is also very similar to that of four clinical isolates of indole-negative cocci previously described (Murdoch *et al.*, 1988). We suggest, therefore, that indole-negative strains of *Ps. asaccharolyticus* occur. If this is correct, the indole reaction would appear to be of little value. The remaining indole-negative, butyrate-producing cocci (originally designated as *Ps. prevotii*) did not produce a consistent pattern. Organisms classified as *Ps. prevotii* and *Ps. tetradius* are almost certainly heterogeneous, as acknowledged by Ezaki *et al.* (1983) in their original description of *Ps. tetradius*; performed enzyme profiles may assist in resolving the taxonomy of this group.

Several representative streptococci were studied because strains of some species are micro-aerophilic (*S. morbillorum* and *S. intermedius*) or even obligately anaerobic (*S. parvulus*). The micro-aerophilic species should not be misidentified as anaerobic cocci because they are resistant to metronidazole; however, the type strain of *S. parvulus* was a small chain-forming coccus, sensitive to metronidazole, and therefore easily confused with *Ps. micros*. This study suggests that *S. parvulus* can be distinguished by means of its enzyme profile.

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