

CLINICAL MICROBIOLOGY

## Evaluation of a phenotypic scheme for the identification of 'butyrate-producing' *Peptostreptococcus* species

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Gram-positive anaerobic cocci (GPAC) are isolated from approximately one quarter of all infections involving anaerobic bacteria. However, studies of the significance of this group of pathogens have been hindered by an inadequate taxonomy and the lack of a valid identification scheme. In the present study, a phenotypic scheme for the identification of 'butyrate-producing' GPAC based on the analysis of volatile fatty acid profiles by gas-liquid chromatography, biochemical profiles (including the use of the rapid ID 32 A commercial kit) and carbohydrate fermentation reactions, was evaluated. The identity of 68 clinical isolates of GPAC was determined by application of the scheme published by Murdoch. The scheme was found to be easy to apply and only four of the test isolates could not be readily assigned to a species or well-defined group. The species most frequently identified in the test collection were *Peptostreptococcus vaginalis*, *P. tetradius* and the  $\beta$ GAL group. A large number of strains was assigned to the heterogeneous 'prevotii/tetradius' group. Some species regarded as being restricted to particular clinical sites were shown to be more widespread than previously thought. The clinical source of the isolates did not show any consistent correlation with species identity.

### Introduction

Gram-positive anaerobic cocci (GPAC) are members of the commensal microflora in a range of body sites [1]. However, GPAC account for 24–31% of all isolates from anaerobic infection and, on occasions, may be isolated in pure culture from infected sites [2–5].

In the past, GPAC have been little studied because of the lack of a sound classification scheme and the recognised heterogeneity of the previously defined species. Most clinical isolates of GPAC are identified to species level within the genus *Peptostreptococcus*. Correct identification of these species is essential to determine whether individual taxonomic groups are associated with specific sites of isolation or disease conditions. Recent taxonomic studies based on molecular approaches such as ribosomal RNA sequencing,

DNA hybridisation and pyrolysis mass spectrometry (PMS) have better defined the taxonomic groups within the GPAC [6–8].

Following the recent reclassification of *P. heliotrinreducens* as *Slackia heliotrinreducens* [9], there are currently 16 species in the genus *Peptostreptococcus*. Three of these species, *P. harei*, *P. octavius* and *P. ivorii* were proposed relatively recently [10]. Two other well-defined groups, the ' $\beta$ GAL' group and the '*trisimilis*' group of strains, have been described, and it has been suggested that these merit species status [11].

It has been shown recently that the GPAC species, as supported by the molecular data, can be discriminated by routine phenotypic methods. Murdoch summarised the differential characteristics of the GPAC [11], including the newly described species, based on volatile fatty acid (VFA) profiles, carbohydrate fermentation reactions and the enzyme profiles obtained with the rapid ID 32 A commercial kit. In particular, the work demonstrated the considerable heterogeneity within the group producing butyric acid as the terminal VFA of glucose metabolism.

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The aim of the present study was to assess the usefulness of the Murdoch scheme [11] and the revised taxonomy for the identification of previously unspiciated strains of butyrate-producing GPAC referred to the PHLS Anaerobe Reference Unit and to determine whether there was any association between species and site of isolation.

## Materials and methods

All strains tested were from the PHLS Anaerobe Reference Unit culture collection, Department of Medical Microbiology and Public Health Laboratory, Cardiff. The clinical source is indicated where known (Table 1). Stock cultures were stored at  $-80^{\circ}\text{C}$  on Microbank beads (Prolab Diagnostics, Cheshire). Strains were subcultured on Fastidious Anaerobe Agar (FAA; Lab M, Bury) supplemented with horse blood 5% and incubated in anaerobic conditions ( $\text{CO}_2$  10%,  $\text{H}_2$  10%,  $\text{N}_2$  80%) at  $37^{\circ}\text{C}$ . Strains were examined according to Gram's stain morphology, growth characteristics, carbohydrate fermentation reactions [12] and VFA profiles determined by gas-liquid chromatography [13]. Strains were also analysed with the rapid ID 32 A identification kit for anaerobes (bioMérieux, Lyon, France) according to the manufacturer's instructions. The bacterial suspension for inoculation of the commercial kit was obtained by harvesting growth from the surface of Columbia blood agar plates (Oxoid) after anaerobic incubation for 48 h to produce a bacterial suspension of McFarland standard 4 turbidity. Identification was based upon visual interpretation of the rapid ID 32 kit results.

## Results

Seven strains selected for study were found not to be butyrate producers and were easily identified as *P. magnus* (5) and *P. micros* (2). Of the remaining 61 butyrate producers, 57 were easily identified according to the scheme and their identities are shown in Table 1. The most frequently identified species were *P. tetradius* (11) and *P. vaginalis* (10). Other groups represented by a large number of test strains included the  $\beta\text{GAL}$  group (9) and the *prevotii/tetradius* group (10). In most cases, VFA profiles, sugar fermentation patterns and enzyme profiles from the commercial kit for each of these strains correlated with the results obtained in the Murdoch study [11]. Minor exceptions included three strains of *P. vaginalis* that produced only weak positive results for ADH. This was reproducible on repeated testing. Four strains yielded profiles which did not correlate with those described in the Murdoch scheme [11]. The profiles obtained are shown in Table 1 and were confirmed by repeated testing on at least two occasions. Optimal bacterial growth was confirmed on each occasion by the demonstration of McFarland standard 4 turbidity. The clinical source of the isolates

did not show any consistent correlation with species identity.

## Discussion

The current study confirms the value of the identification method proposed by Murdoch [11]. The methods were easy to perform in routine diagnostic work and the identification scheme could be readily applied. The majority of test isolates gave consistent results and were identified easily. The scheme, which is based on conventional phenotypic methods and relies heavily on the generation of a biochemical enzyme profile with a commercial kit for species differentiation, has been supported by additional taxonomic studies based on molecular analyses and PMS [6]. The value of such commercial kits can be appreciated not only for rapid identification of known species but also the recognition of novel taxa. 'ADH' and Hare group III were recognised as distinct by the enzyme profiles before formal species identification as *P. vaginalis* and *P. hydrogenalis*.

*P. vaginalis* was proposed as a new species in 1992 on the basis of DNA hybridisation studies and is thought to be one of the more pathogenic species of GPAC [8, 11]. Ten of the test strains were identified as *P. vaginalis* and were easily recognised on the basis of their pre-formed enzyme profiles (PEP). Three strains were consistently only weakly positive for ADH. None of the 10 strains identified as *P. vaginalis* formed indole, a finding which has been reported previously only for a minority of strains. Interestingly, this species has been isolated mainly from subdiaphragmatic infections although, in the present study, strains identified as *P. vaginalis* were isolated from a range of superficial sites (eye and ear) and deep sites including three isolates from the brain and cerebrospinal fluid.

Nine strains were identified as belonging to the ' $\beta\text{GAL}$ ' group. Although regarded as a relatively heterogeneous collection of organisms, the present study has confirmed that members of this group can be differentiated easily from other species on the basis of their PEPs. The range of sites from which the  $\beta\text{GAL}$  strains were isolated is similar to that reported previously and included soft tissue abscesses and infected graft sites.

Eleven isolates produced profiles characteristic of either *P. asaccharolyticus* or *P. harei*. It has been suggested that these species can be easily differentiated on the basis of cell and colony characteristics. Colonies of *P. asaccharolyticus* are reported as being more convex and the cells are of more uniform size. However, these morphological properties may be medium dependent, making them unreliable as a basis for differentiation of the species and it was decided that for these 11 strains such characteristics were not

**Table 1.** Differential characteristics of 'butyrate-producing strains of GPAC\*

Species	Number of isolates	Terminal VFA	Production of				Carbohydrate fermentation reactions					Production of saccharolytic and proteolytic enzymes										
			Indole	Urease	ALP	ADH	Glu	Lac	Raf	Rib	Mne	$\alpha$ GAL	$\beta$ GAL	$\alpha$ GLU	$\beta$ GUR	ArgA	ProA	PheA	LeuA	PyrA	TyrA	HisA
<i>P. vaginalis</i>	10	B	-	-	-/w	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+
$\beta$ GAL group	9	B	-	-	w	-	+	+	-	+	+	-	w/+	-/w	-	+	-	-	+	w/+	-	-
<i>P. tetradius</i>	11	B	-	+	-	-	+	-	-	-	+	-	-	+	+	+	-	w/+	+	w	w	w
<i>P. lacrimalis</i>	3	B	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	w/+	+
' <i>trisimilis</i> ' group	2	B	+	-	-	-	+	w	w	w	+	-	-	-	-	-	-	-	-	+	-	-
		B	+	-	+	-	+	-	-	-	+	-	w	-	-	-	-	-	-	+	-	-
<i>P. indolicus</i>	1	B	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	w	+
<i>P. asaccharolyticus/P. harei</i>	8	b	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	w/-	-	w/-	+/w
	3	b	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	w/-	-	w	+
<i>prevotii/tetradius</i> group	1	b	-	-	-	-	+	-	-	-	w	w	w	+	+	w	-	-	-	+	-	+
	1	b	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	-	+
	1	b	-	-	-	-	w	-	-	-	-	-	+	+	+	+	-	-	-	+	-	w
	1	B	-	+	-	-	w	-	-	-	-	-	w	w	w	+	-	w	w	w	w	-
	1	B	-	w	-	-	+	-	-	-	-	-	w	w	w	+	-	-	-	-	-	w
	1	B	-	-	-	-	+	-	-	-	-	-	-	w	w	+	-	-	+	-	-	-
	1	B	-	-	-	-	w	-	-	-	-	-	w	w	w	+	-	-	-	-	-	+
	1	B	-	-	-	-	-	-	-	-	-	-	-	w	w	+	-	-	w	-	-	+
	1	B	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-	-	-	+	-	-
Unidentified strains	1	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-
	1	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	1	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
	1	B	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+

VFA, volatile fatty acids; ALP, alkaline phosphatase; ADH, arginine dihydrolase; Glu, glucose; Lac, lactose; Raf, raffinose; Rib, ribose; Mne, mannose;  $\alpha$ GAL,  $\alpha$ -galactosidase;  $\alpha$ GLU,  $\alpha$ -glucosidase;  $\beta$ GUR,  $\beta$ -glucuronidase; ArgA, arginine arylamidase (AMD); ProA, proline AMD; PheA, phenylalanine AMD; LeuA, leucine AMD; TyrA, tyrosine AMD; HisA, histidine AMD; -, negative; +, positive; w, weakly positive; B/C, blood culture.

\*Source of isolates: *P. vaginalis*: blood culture (4), CSF (2), ear, eye, brain abscess, thigh wound.  $\beta$ GAL group: blood culture (4), gangrenous tissue, graft, buttock abscess, brain abscess, leg ulcer, graft. *P. tetradius*: blood culture (5), lung, dental cyst, shoulder abscess, placenta, laparotomy wound, episiotomy. *P. lacrimalis*: blood culture, vagina, wound. '*trisimilis*' group: blood culture (2). *P. indolicus*: blood culture. *P. asaccharolyticus/P. harei*: blood culture (8), urinary tract infection, abscess, brain abscess. *prevotii/tetradius* group: blood culture (6), pus-orthopaedic, wound axilla, knee fluid, unknown. Unidentified strains; blood culture (2), neck abscess, abscess.

sufficiently marked to allow speciation. Further methods would be required for accurate identification, such as PMS or 16S rDNA analysis.

The recently described species *P. lacrimalis* is strongly proteolytic, and three test strains conformed to its profile. The two strains previously studied by Murdoch were both isolated from the eye [11], whereas in the present study *P. lacrimalis* strains were encountered in a range of clinical sites (vagina, blood culture and a wound). Such a pattern of isolation may indicate that the species is more widespread than at first thought. Only one test strain was identified as *P. indolicus*. This is in keeping with previous studies which report that, although an important veterinary pathogen, the species is rarely isolated from human sources [11]. The 'trisimilis' group is a well-defined group, distinct from *P. hydrogenalis*. Two test species conformed to the profile of this little studied group.

In the past it has been reported that *P. prevotii* and *P. tetradius* were two of the species of GPAC most frequently detected in clinical material [2–4] and both species are regarded as constituents of the normal vaginal flora [14]. However, it has since been realised that these 'butyrate-producers' represent a very heterogeneous collection of strains. Strains conforming to the strict definition of the *P. prevotii* type strain have been isolated from pathological specimens only occasionally. No strains similar to the type strain of *P. prevotii* were identified in the test collection. However, a large number of strains yielded profiles similar to that of the *P. tetradius* type strain. All were strongly urease positive and strongly saccharolytic and were isolated from a diverse range of sites including a dental cyst and the lung. This is somewhat surprising as, recently, most 'butyrate producers' have been assigned to the less well-defined *prevotii/tetradius* group. Ten of the test strains fell into this latter group and the profiles were more variable than those of the strains that conformed to the profile for the *P. tetradius* type strain. *P. prevotii* and *P. tetradius* have been shown to cluster closely together by both biochemical and PMS analysis and are now regarded as a heterogeneous collection of saccharolytic organisms [11]. The present study supports the heterogeneity of the group and the variable profiles of test strains allocated to this group are listed in Table 1.

Some of the currently recognised GPAC species were not represented in the test collection, e.g., *P. lactolyticus* and *P. octavius*. This is perhaps not surprising because previous work has been based on only small numbers of isolates representing these groups, suggesting that these species are encountered only rarely in clinical specimens.

The identity of the four test strains that yielded unrecognised profiles is unknown and it is possible that these may represent new taxonomic groups or

phenotypic variants of existing species. These strains merit further study, for example, the analysis of 16S rDNA sequences would be useful.

Since the availability of a valid classification scheme incorporating the new species and groups, there have been few reports on the frequency of isolation of the individual new species. It should be stressed that the test collection in this case may not be representative of the frequency of isolation of individual species, because it contains many isolates which are referred for identification and the collection is therefore subject to bias. Furthermore, it has been suggested that until all species have been phylogenetically validated, reports of incidence should be reported with caution [11]. Nevertheless, it is interesting to observe the frequency of identification of the individual taxa. The most frequently identified species within this test collection of 'butyrate-producers' were *P. vaginalis*, *P. tetradius*,  $\beta$ GAL group, *P. asaccharolyticus/P. harei* and the heterogeneous *prevotii/tetradius* group.

In summary, the Murdoch scheme [11] was found to be easy to apply to the test collection of 'butyrate-producing' GPAC and the majority of isolates were assigned to well-defined groups or species. The scheme is likely to be of value in the further investigation of this heterogeneous group of pathogens.

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