Dehydrogenase Patterns in the Taxonomy of *Bacteroides*

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The malate dehydrogenase (MDH) electrophoretic mobilities of 128 strains of bacteroides belonging to 17 species, including three subspecies of *Bacteroides melaninogenicus* and two subspecies of *Bacteroides ruminicola*, were examined. Amongst the pigmented bacteroides, the migration of this enzyme correlated well with recognized taxa, and only one strain, VPI 9085 was clearly different. Other species such as *B. oralis*, *B. buccalis*, *B. denticola*, *B. pentosaceus*, *B. biviaus*, *B. disiens* and *B. ruminicola* were delineated by the combined use of MDH and glutamate dehydrogenase. Forty-three strains belonging to the '*B. fragilis* group' differed from the above species in possessing glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, and reference strains as well as fresh isolates were assigned to the correct species by the mobility pattern of these two enzymes.

Other properties of MDH such as the pH optima for the oxidation of malate or the reduction of oxaloacetate were of limited taxonomic value. However, the alkaline stability of this enzyme at pH 9, 10 and 11 clearly differentiates the saccharolytic from the non-saccharolytic species of pigmented bacteroides with the latter showing highly stable enzymes with a half life greater than 50 min.

INTRODUCTION

Many physiological tests used in bacteriology depend on the presence of particular enzymes. The measurement of enzyme activities within cells has also been used in taxonomy (De Ley, 1962, 1968; Bascombe, 1980) and the presence of key enzymes, and metabolic pathways dependent upon them is fundamental to the production of distinctive patterns of metabolic end-products (Sokatch, 1969). If an enzyme is present within all strains of a group of bacteria then its activity is of no taxonomic value (Norris, 1968), but its molecular properties can be detectably different in various taxa. One easily detectable difference is electrophoretic mobility (Shaw, 1965), but other properties such as molecular weight and coenzyme specificity may also be used (Weitzman, 1980). We have reported differences in malate dehydrogenase (MDH) mobilities between the three formerly recognized subspecies of *Bacteroides melaninogenicus* (Williams *et al.*, 1975). Malate dehydrogenase is not found in fusobacteria but its presence was confirmed in a larger study of the black-pigmented bacteroides (Shah *et al.*, 1976), although heterogeneity of both MDH mobility and DNA base composition was evident. The only available reference strain of *B. melaninogenicus* subsp. *melaninogenicus* used initially (VPI 9085) was later shown to be quite different to other strains of this subspecies (Shah *et al.*, 1976), and was listed as a species *incertae sedis* (Shah, 1981). The value of MDH mobility was supported by the correspondence between enzyme mobility and DNA base composition (Shah *et al.*, 1976) which revealed two distinct taxa for which new species names have been proposed (Coykendall *et al.*, 1980; van Steenberg *et al.*, 1981). This paper describes the mobility of MDH in a wider group of pigmented bacteroides strains, and the stability and pH optima of this enzyme, together with the electrophoretic patterns of other enzymes in both pigmented and non-pigmented bacteroides.

METHODS

Bacterial strains. The 128 strains of *Bacteroides*, mostly of human oral origin, used are listed in Table 1.

Growth of cultures and preparation of cell-free extracts. Strains were grown in liquid media as described previously (Shah *et al.*, 1976). For electrophoresis of enzymes cells were grown anaerobically under 10% carbon dioxide, 20% hydrogen in nitrogen for 3 d at 37 °C on plates containing defibrinated horse blood (Wellcome), 5% (w/v) and Blood Agar Base no. 2 (Oxoid), 4% (w/v).

Table 1. *Strains used and their sources*

Strain no.	Source (received from)*	Site of isolation
<i>Bacteroides asaccharolyticus</i>		
VPI 4198	VPI	Empyema
VPI 4199	V. L. Sutter	Human faeces
NCTC 9337	NCTC	Infected haemorrhoids
B 536	V. L. Sutter	Human faeces
B 537	V. L. Sutter	Human leg lesion
B 477	V. L. Sutter	Human faeces
<i>Bacteroides bivius</i>		
VPI 6822	VPI	Endometrium
VPI 6318	VPI	Vaginal swabs
' <i>Bacteroides bivius</i> -like'		
5540	E. M. Barnes	Clinical specimen
7880	E. M. Barnes	Clinical specimen
<i>Bacteroides disiens</i>		
VPI 7852	VPI	Draining stomach infusion
VPI 8057	VPI	Bartholin abscess
<i>Bacteroides distasonis</i>		
ATCC 8503	ATCC	Faeces
three clinical isolates	London Hospital	
<i>Bacteroides eggerthii</i>		
NCTC 11155	B. I. Duerden	NK
<i>Bacteroides fragilis</i>		
NCTC 8560	NCTC	Appendectomy
NCTC 9343	NCTC	Appendix abscess
13 clinical isolates	London Hospital	
<i>Bacteroides gingivalis</i>		
W50	H. Werner	Clinical specimen
W83		
P157		
P210	P. Kho	Periodontal plaque
WPH15		
WPH35	W. P. Holbrook	Dental plaque
WPH34		
<i>Bacteroides melaninogenicus</i>		
subsp. <i>intermedius</i>		
NCTC 9336	NCTC	Vincent's gingivitis
NCTC 9338	NCTC	Empyema
B9	T. Hofstad	Gingival mucosa
B18		
B22		
B23		
N275-70A	D. W. Lambe	Oral specimen
3b	W. Loesche	
D13a-f1	J. Carlsson	Dental root canal
G11a-d2		
JP1	M. M. Garcia	Cattle horn abscess
2845	V. L. Sutter	Post gastrectomy wound
2846	V. L. Sutter	Lung aspirate
2849	V. L. Sutter	Trans-tracheal aspirate
P209	P. Kho	Peridontal plaque
P156		

Table 1 (*continued*)

Strain no.	Source (received from)*	Site of isolation
T588 T584 M107 LH107 LH45 LH100 LH116 AM1726 S1 S4 S9 S12 S20 S23 S24	Own isolates	Dental plaque
<i>Bacteroides melaninogenicus</i>		
subsp. <i>levii</i>		
JP2	M. M. Garcia	Cattle horn abscess
Lev 1	M. Lev	Rumen contents
<i>Bacteroides melaninogenicus</i>		
subsp. <i>melaninogenicus</i>		
VPI 9085	E. Cato	Gingival crevice
VPI 4196	E. Cato	Human sputum
WAL 2728	V. L. Sutter	Faecal fluid—ruptured bowel
WAL 2721	V. L. Sutter	Trans-tracheal aspirate
WAL 2724	V. L. Sutter	Trans-tracheal aspirate
WAL 3150	V. L. Sutter	Peritoneal fluid
N138-71	D. W. Lambe	NK
N334-71H	D. W. Lambe	Oral specimen
23K9	C. B. Sabiston	Peridontal abscess
S2 S5 S13 S14 S18 S21 S22 S25 S26	Own isolate	Dental plaque
<i>Bacteroides oralis</i>		
ATCC 33269	VPI	Dental plaque
' <i>Bacteroides oralis</i> -like'		
VPI 8096D	VPI	Umbilical drainage
HS4	Own isolate	Dental plaque
1210 1221	S. S. Socransky	Dental plaque
<i>Bacteroides ovatus</i>		
ATCC 8483	London Hospital	NK
2 clinical isolates		
<i>Bacteroides ruminicola</i>		
subsp. <i>brevis</i>		
GA33 118B B5	M. J. Latham	Rumen
<i>Bacteroides ruminicola</i>		
subsp. <i>ruminicola</i>		
R1 R2 R5	M. J. Latham	Rumen

Table 1 (*continued*)

Strain no.	Source (received from)*	Site of isolation
<i>'Bacteroides ruminicola-like'</i>		
NP333	Own isolate	Dental plaque
WPH61	W. P. Holbrook	Dental plaque
J1	W. J. Loesche	Dental plaque
<i>Bacteroides splanchnicus</i>		
NCTC 10826	B. I. Duerden	NK
<i>Bacteroides thetaiotaomicron</i>		
NCTC 10582	NCTC	Faeces
W910	H. Werner	NK
10 clinical isolates	London Hospital	
<i>Bacteroides vulgatus</i>		
WBM 137	H. Werner	NK
6 clinical isolates	London Hospital	

NK, Not known.

* VPI, Virginia Polytechnic Institute; NCTC, National Collection of Type Cultures; ATCC, American Type Culture Collection.

Cells were harvested from liquid culture (Shah *et al.*, 1976) or from blood agar plates (three per strain) by suspending the cells in 0.1 M-HEPES buffer pH 8.0 (BDH). The cell suspension was shaken with Ballotini beads (Grade 12, Jencons Ltd.) for 15 min in a Mickel Tissue disintegrator (Mickle Engineering Ltd, Gomshall, Surrey) at 4 °C. Beads, cell debris and unbroken cells were removed by centrifugation at 37000 *g* for 15 min and the supernatant, containing 20–30 mg protein ml⁻¹, was used for studies of the enzymes.

Electrophoresis and staining of dehydrogenases. Cellolog strips 5.7 × 14 cm (Reeve Angel Scientific Ltd) were soaked in 0.04 M-barbitone-acetate buffer pH 8.6 30 min before use. Cell-free extracts were loaded (1.2 µl) at the origin line and electrophoresed for 1 h at 10 volts per cm length of the gel upon a supporting block of the LKB Multiphor cooled by water at 10 °C. Enzymes were stained as follows: malate dehydrogenase (MDH) according to Williams *et al.* (1975); glutamate dehydrogenase (GDH) by the method for MDH except that glutamate was substituted for malate; glucose-6-phosphate dehydrogenase (G6PDH), following the procedure of Williams & Bowden (1968); and 6-phosphogluconate dehydrogenase (6PGDH) by the method for G6PD except that 6-phosphogluconate was substituted for glucose-6-phosphate. For MDH and GDH enzymes NAD was used whereas NADP and NAD were used for G6PD and 6PGD.

Measurement of enzyme activity. All enzyme assays were carried out at 25 °C in a Gilford model 240 spectrophotometer. Bacterial extracts prepared as above (for electrophoresis) were assayed for enzyme activity by following the formation of the reduced pyridine nucleotide at 340 nm according to Reeves *et al.* (1971). Reactions were carried out in 3 ml volumes at pH 7.5 in 0.1 M-HEPES buffer and contained 0.49 mM-NAD. Substrate concentrations were 5 mM-L-malate, 3.5 mM-glutamate, 2.2 mM-glucose 6-phosphate or 1.8 mM-6-phosphogluconate.

Determination of pH optimum. The pH optimum for the oxidation of L-malate was determined by the above procedure. The reduction of oxaloacetate to malate was assayed by following the disappearance of NADH at 340 nm. The reaction mixtures contained 0.1 M buffer, 0.24 mM-NADH and 2.5 mM-oxaloacetate. The buffers used were MES, MOPS, HEPES, CHES, CAPS and Tricine (BDH).

Determination of the half inactivation times of malate dehydrogenase in alkaline buffers. Cell-free extracts were incubated separately in buffers at pH 9, 10 or 11 for up to 50 min in a 37 °C waterbath. Samples (0.1 ml) were removed at timed intervals and assayed for MDH activity (reduction of oxaloacetate) as described above. The percentage enzyme activity relative to the initial reaction of extracts not treated with alkaline buffers was calculated. The time taken for a 50% loss of activity (*t*_{0.5}, the half inactivation time) was determined graphically.

RESULTS AND DISCUSSION

Malate dehydrogenase mobilities were determined by cooled electrophoresis, which gave lower mobilities than those reported earlier (Shah *et al.*, 1976). The results for 64 strains of pigmented bacteroides (Table 2) confirm that strains now called *Bacteroides gingivalis* (Coykendall *et al.*, 1980) and previously recognized as the low percentage G + C group of *B. melaninogenicus* subspecies *asaccharolyticus* (Shah *et al.*, 1976), have a lower MDH mobility than most strains of *Bacteroides asaccharolyticus* (Finegold & Barnes, 1977), which was previously recognized as the high G + C group of *B. melaninogenicus* subspecies *asaccharolyticus* (Shah *et*

Table 2. Mobilities of malate dehydrogenase in strains of pigmented *Bacteroides*

Strain	Relative mobility* (cm)
<i>Bacteroides gingivalis</i>	
P157, P210	2.7
WPH15, WPH35	2.8
WPH34	2.9
W50, W83	3.1
<i>Bacteroides asaccharolyticus</i>	
VPI 4198	3.2
B536, B537, NCTC 9337	4.3
B477, VPI 4199	4.4
<i>Bacteroides melaninogenicus</i>	
subsp. <i>intermedius</i>	
T588, T584, M107, NCTC 9336, S3, NCTC 9338	4.0
P209, P156	4.1
B9, B18, 2845, 2846, LH107, AM1726	4.2
S9, S20, S23, S24, S12, N275-70A, S1, S4	4.3
2849	4.5
B22, B23, JP1, LH45, LH100, 3b, D13a-f1	4.6
G11a-d2, LH116	4.7
<i>Bacteroides melaninogenicus</i>	
subsp. <i>melaninogenicus</i>	
VPI 9085	3.2
VPI 4196	5.1
WAL2728, WAL2721, WAL2724, 23K9, S14	5.2
WAL 3150, S25, S21, S22	5.3
N138-71, N334-71H	5.4
S2	5.8
S5	5.9
S13	6.0
S26	6.1
S18	6.2
<i>Bacteroides melaninogenicus</i>	
subsp. <i>levii</i>	
JP2	2.7
Levl	3.9

* All MDH mobilities were measured relative to the enzyme of strain T588.

al., 1976). The large group of 31 strains of *B. melaninogenicus* subspecies *intermedius* show a continuous spectrum of migration distances. This range is shown as a broad band in Fig. 1. The electrophoresis of this enzyme does not distinguish these strains from those of *B. asaccharolyticus*. Amongst 18 strains of *B. melaninogenicus* subspecies *melaninogenicus*, MDH showed two mobility clusters, the larger 5.1–5.4 cm, and the smaller 5.8–6.2 cm. One strain (VPI 9085) has an abnormally low enzyme migration of 3.2 cm and high DNA base composition (Shah *et al.*, 1976) which indicates that it cannot be a member of this taxon despite its physiological properties. Like *B. asaccharolyticus*, the two strains of *B. melaninogenicus* subspecies *levii* have similar DNA base compositions (van Steenberg *et al.*, 1979) and a high DNA–DNA homology with each other (van Steenberg & de Graaff, 1979), but different malate dehydrogenase mobilities. Despite heterogeneity within them, the subspecies *intermedius*, *levii* and *melaninogenicus* are all sufficiently different to warrant elevation to species rank (Williams & Shah, 1980).

Five strains received, or initially diagnosed, as *B. oralis* had three patterns of MDH and GDH mobility. *B. oralis* ATCC 33269, the neotype strain (Skerman *et al.*, 1980), is the only authentic strain of this species examined. The two strains now called *Bacteroides buccalis* and the two called *Bacteroides denticola* (Shah & Collins, 1981) resemble ATCC 33269 physiologically, but

Fig. 1. Electrophoretic patterns of malate dehydrogenase (hatched, faint bands with broken outlines) and glutamate dehydrogenase (unhatched bands) amongst bacteroides strains.

differ in their enzyme patterns (Table 3 and Fig. 1), lipid composition (Shah & Collins, 1980), DNA base composition and DNA homology (Shah *et al.*, 1981). Two strains which were received as *B. oralis* (5540 and 7880) show identical MDH and GDH mobilities to reference strains of *Bacteroides bivia* (Table 3). Similar DNA base composition and high DNA homology amongst these four strains confirm this relationship (Shah *et al.*, 1981). *Bacteroides disi* phenotypically resembles *B. bivia* except in the fermentation of lactose and galactose. DNA homology (Holdeman & Johnson, 1977) distinguishes these two species, however the combined use of MDH and GDH electrophoresis (Table 3 and Fig. 1) provides a simple and reliable method of identifying such strains.

Strains of oral origin which were considered to be *B. oralis*, but which ferment pentoses and contain 50% G + C were described as *B. ruminicola* subspecies *brevis* (personal communication L. V. Holdeman). These three strains (NP 333, WPH 61 and J1, Table 3) have enzyme mobilities which are quite distinct from those of reference strains of either subspecies of *B. ruminicola* (Fig. 1), and they have been allocated to *Bacteroides pentosaceus* (Shah & Collins, 1981) on other chemotaxonomic criteria.

The stimulation of growth by bile (Holdeman *et al.*, 1977) or sodium taurocholate (Duerden *et*

Table 3. Mobilities of malate dehydrogenase and glutamate dehydrogenase in putative strains of *Bacteroides oralis*, *Bacteroides ruminicola*, *Bacteroides bivius* and *Bacteroides disiens*

Strain		Relative mobility in Cellogel* (cm)	
		MDH	GDH
<i>Bacteroides oralis</i>			
ATCC 33269		4.1	5.2
' <i>Bacteroides oralis</i> -like' strains			
VPI 8906D		5.3	4.1
HS4		5.3	4.2
1210		4.2	3.4
1221		4.1	3.4
<i>Bacteroides bivius</i>			
VPI 6822		5.8	3.1
VPI 6318		5.8	3.1
' <i>Bacteroides bivius</i> -like' strains			
5540		5.8	3.1
7880		5.8	3.1
<i>Bacteroides disiens</i>			
VPI 7852		4.4	1.5
VPI 8057		4.2	1.5
<i>B. ruminicola</i>			
subsp. <i>brevis</i>	GA33	5.9	5.1
	118B	5.8	5.2
	B5	6.0	5.1
subsp. <i>ruminicola</i>	R1	5.7	4.7
	R2	5.4	4.6
	R5	5.8	4.7
Oral isolates, ' <i>B. ruminicola</i> -like'			
NP333		3.9	4.8
WPH 61		3.7	4.8
J1		3.9	4.7

* All MDH mobilities were measured relative to the enzyme of strain T588.

al., 1976) is a unique property of the '*B. fragilis* group of species'. Forty-three strains (nine reference and thirty-four recent clinical isolates) which belong to this group have been examined enzymologically. Fifteen strains of *B. fragilis*, twelve of *Bacteroides thetaiotaomicron* and three of *Bacteroides ovatus* all had species-specific patterns of MDH that comprised three bands (Fig. 1). *Bacteroides distasonis*, *Bacteroides vulgatus*, *Bacteroides eggerthii* and *Bacteroides splanchnicus* strains all had single MDH bands of such similar mobility as to render them indistinguishable. Amongst these four species the electrophoresis of GDH produced similar fast migrating bands in *B. distasonis* and *B. vulgatus*, while *B. eggerthii* and *B. splanchnicus* enzyme moved less than half this distance (Table 4). Enzyme assay of all 43 strains revealed the presence of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Table 4) enzymes that were universally absent in all other bacteroides species tested. It seems, therefore, that the presence of these two enzymes of the pentose phosphate pathway are diagnostic of species of the '*B. fragilis* group'. The magnitude of the specific enzyme activity of all four dehydrogenases (Table 4) is not significantly different between these species. It was not possible to identify a strain as belonging to a species using the mobility of either G6PDH or 6PDGH because of ambiguities, e.g. both *B. eggerthii* and *B. splanchnicus* have G6PDH of the same migration rate. However, taken together the electrophoresis of both enzymes provided unambiguous identification (Table 4). Thirty fresh clinical isolates were identified by these species-specific electrophoretic patterns before the

Table 4. *Enzyme mobilities and activities amongst the B. fragilis group of species*

Organism	Strain	Mobility in Cellogel (cm)*				Activity [nmol (mg protein) ⁻¹ min ⁻¹]			
		MDH	GDH	6PGDH	G6PDH	MDH	GDH	6PGDH	G6PDH
<i>B. fragilis</i>	NCTC 8560	0.0, 2.9, 3.8	5.9	2.1	3.3	304.6	236.9	84.6	194.6
	NCTC 9343	0.0, 2.9, 3.8	5.9	2.2	0.0, 3.4	85.5	222.3	106.2	153.9
	13 strains	0.0, 2.6-3.0, 3.6-4.0	5.8-6.1	2.0-2.4	2.9-3.6	-	-	-	-
<i>B. thetaiotaomicron</i>	NCTC 10582	0.0, 4.1, 4.6	5.0	1.2	3.7	225.4	127.7	45.1	82.9
	W 910	0.0, 4.1, 4.6	5.0	1.2	3.8	111.9	292.8	137.8	48.2
	10 strains	0.0, 3.8-4.2, 4.6-4.7	4.9-5.2	0.9-1.4†	3.6-4.0	-	-	-	-
<i>B. distasonis</i>	ATCC 8503	3.5	5.1	2.5, 2.9	3.7, 4.1	180.8	93.8	100.5	154.1
	3 strains	3.3-3.6	4.9-5.1	2.3-2.5, 2.8-2.9	3.5-3.7, 4.0-4.2	-	-	-	-
<i>B. ovatus</i>	ATCC 8483	3.7, 4.3, 5.0	5.6	0.5, 1.0	1.5, 2.9	65.2	36.2	57.9	18.8
	2 strains	3.5, 4.8	5.6-5.9	1.0	3.0-3.4‡	-	-	-	-
<i>B. vulgatus</i>	WBM 137	3.8	4.9	2.8	0.0, 4.0, 4.7, 5.0	301.4	562.7	26.8	57.6
	6 strains	3.7-3.9	4.5-4.9	2.3-2.8	2.5-2.9, 3.5-4.0, 4.8-5.2	-	-	-	-
<i>B. eggerthii</i>	NCTC 11155	3.7	1.6	1.4	1.7	167.1	525.3	39.8	28.6
<i>B. splanchnicus</i>	NCTC 10826	3.7	1.4	2.6, 3.5	1.7	136.7	482.3	48.2	32.2

* All enzyme mobilities were measured relative to the major band of strain NCTC 8560.

† Two strains 111 and S6 contained additional 6PGDH bands at 5.0-5.2 and 5.8-6.0 cm.

‡ One strain 112 had an additional G6PDH band at 5.2 cm.

Table 5. Time of half inactivation of malate dehydrogenase of pigmented *Bacteroides* in alkaline buffers at 37 °C

Strain	$t_{0.5}$ (min)*		
	pH 9	pH 10	pH 11
<i>B. asaccharolyticus</i> NCTC 9337, B536, B537	∞	> 50	> 50
<i>B. gingivalis</i> W50, W83, P157, P210	∞	> 50	> 50
<i>B. melaninogenicus</i> subsp. <i>levii</i> JP2, Lev I	∞	> 50	> 50
<i>B. melaninogenicus</i> subsp. <i>intermedius</i> S4	24	20	2
P209	31	24	8
P156	43	40	9
P12	39	32	9
N275	> 50	40	16
S23	> 50	48	18
S3	> 50	50	26
S24	> 50	37	27
S20	> 50	41	28
S9	> 50	> 50	34
T588	> 50	> 50	42
<i>B. melaninogenicus</i> subsp. <i>melaninogenicus</i> WAL2728	4.0	2.0	0.5
S5	7.0	5.0	0.5
VPI9085	5.5	3.0	0.5
S2	7.0	2.5	0.5
S13	8.0	2.5	0.5
S18	8.0	4.0	0.5
S14	8.0	3.5	0.5
S25	7.0	3.0	1.0
S26	9.5	5.0	1.5

* ∞ indicates no loss of enzyme activity was detected in 50 min and > 50 indicates the loss of activity was linear but the extent was so small as to render the estimation of $t_{0.5}$ inaccurate.

clinical laboratory had examined them. In every case the identification by electrophoresis was confirmed by more conventional tests (Holdeman *et al.*, 1977).

The pH optimum for the reduction of oxaloacetate by malate dehydrogenase in cell-free extracts of bacteroides was found to be pH 8.4 for *B. gingivalis* strain W 83 and *B. melaninogenicus* subspecies *intermedius* strain T 588, but pH 7.5 for *B. melaninogenicus* subspecies *melaninogenicus* strain WAL 2728. Such examinations were of limited taxonomic importance; however, the stability of MDH at pH 9, 10 and 11 (Table 5 and Fig. 2) was of greater significance. This enzyme from *B. asaccharolyticus*, *B. gingivalis* and *B. melaninogenicus* subsp. *levii* was highly stable in these alkaline buffers. Strains of *B. melaninogenicus* subspecies *intermedius* contained MDH that was less stable at pH 10 and 11, and using the latter pH the half life of the enzyme lay between 2 and 42 min. With pH 11, *B. melaninogenicus* subspecies *intermedius* strains were readily distinguished from those of the asaccharolytic species. The malate dehydrogenase of *B. melaninogenicus* subspecies *melaninogenicus* was the least stable of all. Any of the three pH values used could distinguish a strain of this subspecies (Table 5) although inactivation at pH 11 was so rapid as to make the determination of half-life inaccurate. This test may be carried out on cells harvested from a single blood agar plate and can be completed within 2 h, and several strains may be examined in parallel. Susceptibility to alkaline denaturation parallels the metabolic properties in that saccharolytic potential is associated with instability of malate dehydrogenase, whereas asaccharolytic bacteroides have stable enzymes.

Fig. 2. The alkaline inactivation of malate dehydrogenase: ●---●, pH 9.0; ●, pH 10.0; ▲, pH 11.0. Upper graphs: *Bacteroides melaninogenicus* subsp. *intermedius* strains T588 and S24. Lower graphs: negligible inactivation of *Bacteroides gingivalis* enzyme of strains W83 and P157 at pH 11 and rapid inactivation of enzyme of *Bacteroides melaninogenicus* subsp. *melaninogenicus* strains VPI 4196 and S21. The subspecies of the S strains were first identified by the stability of MDH to alkali, and subsequently confirmed.

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