A Numerical Taxonomic Study of *Actinobacillus*, *Pasteurella* and *Yersinia*

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A numerical taxonomic study of strains of *Actinobacillus*, *Pasteurella* and *Yersinia*, with some allied bacteria, showed 23 reasonably distinct groups. These fell into three major areas. Area A contained species of *Actinobacillus* and *Pasteurella*: *A. suis*, *A. equuli*, *A. lignieresii*, *P. haemolytica* biovar A, *P. haemolytica* biovar T, *P. multocida*, *A. actinomycetemcomitans*, '*P. bettii*', '*A. seminis*', *P. ureae* and *P. aerogenes*. Also included in A was a composite group of *Pasteurella pneumotropica* and *P. gallinarum*, together with unnamed groups referred to as 'BLG', 'Mair', 'Ross' and 'aer-2'. Area B contained species of *Yersinia*: *Y. enterocolitica*, *Y. pseudotuberculosis*, *Y. pestis* and a group 'ent-b' similar to *Y. enterocolitica*. Area C contained non-fermenting strains: *Y. philomiragia*, *Moraxella anatipestifer* and a miscellaneous group 'past-b'. There were also a small number of unnamed single strains.

INTRODUCTION

The genera *Actinobacillus* and *Pasteurella* are very similar, and are closely related to *Haemophilus*. Together they form the HPA group (recently reviewed in Kilian *et al.*, 1981). We here present findings on *Actinobacillus* and *Pasteurella* to extend earlier work on *Haemophilus* (Broom & Sneath, 1981) and we have included some data on *Yersinia*, which has historically been considered an allied group. Names that are not validly published [because they are not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) or have not been validly published since then] are 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 culture conditions. Except when stated otherwise all cultures were incubated at 37 °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 McFarland scale. Tests were read after 2 d unless otherwise stated, but API galleries were read after 24 h.

Colonial and cellular morphology. Strains grown on BMA with horse blood (7% v/v) for 24 h were examined for colonial features and slimy colonies recorded. Haemolysis of sheep blood (7% v/v) in BMA was recorded after 24 and 48 h as greening, complete haemolysis, or no haemolysis. Heat-fixed films of 24 h horse blood BMA cultures were stained by Gram's stain (Cowan, 1974), and sizes measured with an eyepiece micrometer. The depth of staining, presence of mucus and extent of bipolar staining were recorded. Motility was examined in hanging drops from 24 h cultures in BMB at 35 °C, and from 48 h cultures in BMB containing 0.25% (w/v) agar at 25 °C and 35 °C.

Growth at different temperatures and resistance to heat. Strains were grown in divided dishes (Replidishes; Sterilin) on BMA, and visible growth was recorded after 2 d at 4 °C, 10 °C, 15 °C and 45 °C. Resistance to heat was

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

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

Strain no.	Name as received	Source and collection no.†	Comments‡
Phenon 1: <i>Actinobacillus suis</i>			
A1	<i>A. suis</i>	Mair 1688/72	Piglet. NCTC 10840; strain 3, Mair <i>et al.</i> (1974)
A2	<i>A. suis</i>	Mair 13443/72	Piglet. NCTC 10843; strain 5, Mair <i>et al.</i> (1974)
A23	<i>A. suis</i>	Mair	Frederiksen P392
A22	<i>A. suis</i>	Mair	Frederiksen P391
A24	<i>A. suis</i>	Mair	Frederiksen P393
A25	<i>A. suis</i>	Mair	Frederiksen P247
A3	<i>A. suis</i>	LPHL 15296/73	Piglet lung abscess
A53	<i>A. suis</i>	NCTC 10840	Same strain as A1
A71	<i>A. suis</i>	Ross 7038	Source not recorded
A21	<i>A. suis</i>	Mair	Frederiksen P390
A17	<i>A. suis</i>	Mair	Frederiksen P379
A18	<i>A. suis</i>	Mair	Frederiksen P380
A7	<i>A. suis</i>	Mair 1689/72	CEN. Piglet. NCTC 10841; strain 4, Mair <i>et al.</i> (1974)
A20	<i>A. suis</i>	Mair	Frederiksen P385
A52	<i>A. suis</i>	NCTC 10843	Same strain as A2
A5	<i>A. suis</i>	LPHL 18808/73	Piglet
A8	<i>A. suis</i>	LPHL 927/73	Piglet septicaemia
A63	<i>A. lignieresii</i>	ATCC 15557	Swine
A54	<i>A. suis</i>	NCTC 10841	Same strain as A7
A16	<i>A. suis</i>	Mair	Frederiksen P378
A19	<i>A. suis</i>	Mair	Frederiksen P381
A70	<i>A. suis</i>	Ross 1388	Source not recorded
A64	<i>A. lignieresii</i>	ATCC 15558	Swine
A4	<i>A. suis</i>	LPHL 25785	Source not recorded
A6	<i>A. suis</i>	LPHL 18809/73	Piglet
A9*	<i>A. suis</i>	LPHL 928/73	Piglet septicaemia
A219*	<i>A. suis</i>	LPHL 20392/79	Swine
A100*	<i>A. suis</i>	LPHL 32452/79	Source not recorded
A153	<i>Actinobacillus</i> sp.	Cutlip 0164	Swine tonsil. D1895
A226*	<i>A. suis</i>	LPHL 27894/79	Piglet
A267*	<i>A. suis</i>	LPHL 18808/73	Replicate of A5
A274*	<i>Actinobacillus</i> sp.	Cutlip 0186	Swine tonsil. D1894
A151	<i>Actinobacillus</i> sp.	Cutlip B15	Swine spleen Cutlip <i>et al.</i> (1972), D1893
A152	<i>Actinobacillus</i> sp.	Cutlip 0186	Replicate of A274
A154	<i>Actinobacillus</i> sp.	Cutlip 0203	D1896
A259*	<i>A. suis</i>	LPHL 9339/78	Horse
A260*	<i>Actinobacillus</i> sp.	LPHL 13028/78	Horse
A261*	<i>Actinobacillus</i> sp.	LPHL 13572/78	Mare
Phenon 2: <i>Actinobacillus equuli</i>			
A26*	<i>A. equuli</i>	NCTC 8529	Foal. TAL, ATCC 19392, D1528, P157, J19
A36*	<i>A. equuli</i>	NCTC 8987	Foal septicaemia
A37*	<i>A. equuli</i>	NCTC 9435	Foal kidney
A34*	<i>A. equuli</i>	NCTC 8794	Foal septicaemia
A31	<i>A. equuli</i>	NCTC 8529	Replicate of A26
A32	<i>A. equuli</i>	NCTC 3365	Foal nephritis
A33	<i>A. equuli</i>	NCTC 8644	Foal kidney abscess. P238
A35	<i>A. equuli</i>	NCTC 8795	CEN. Foal septicaemia
A255*	<i>A. equuli</i>	LPHL 40978/76	Swine
A256*	<i>A. equuli</i>	LPHL 9680/77	CEN. Foal
A257*	<i>A. equuli</i>	LPHL 19411/77	Swine
A258*	<i>A. equuli</i>	LPHL 209/78	Swine
A264*	<i>A. equuli</i>	NCTC 8795	Replicate of A35
Phenon 3: Satellite			
A142	<i>Pasteurella</i> sp.	LPHL 37706/74	D1884. Lung, tamarin monkey
Phenon 4: 'BLG'			
A82	<i>Pasteurella</i> sp.	NCTC 10547	Bovine lymphangitis, India
A83	<i>Pasteurella</i> sp.	NCTC 10548	CEN. Bovine lymphangitis, India
A84*	<i>Pasteurella</i> sp.	NCTC 10549	Bovine lymphangitis, India
Phenon 5: 'Mair'			
A10*	<i>Pasteurella</i> sp.	LPHL 5143/70	CEN. Pig foetus. NCTC 10699, D1187
A12*	<i>Pasteurella</i> sp.	LPHL 14679/70	Swine
A14*	<i>Pasteurella</i> sp.	LPHL 34152/70	Swine
A11*	<i>Pasteurella</i> sp.	LPHL 7385/70	Pig uterus. NCTC 10700

Table 1 (continued)

Strain no.	Name as received	Source and collection no.†	Comments‡
A179*	<i>Pasteurella</i> sp.	LPHL 9801/75	Swine
A13	<i>Pasteurella</i> sp.	LPHL 18286/70	Swine
A278*	<i>Pasteurella</i> sp.	LPHL 14679/70	Replicate of A12
A15	<i>Pasteurella</i> sp.	LPHL 34310/72	Piglet
A204	<i>Pasteurella</i> sp.	LPHL 49605/78	Swine
A212	<i>Pasteurella</i> sp.	LPHL 4376/79	Prairie marmot
Phenon 6: <i>Actinobacillus lignieresii</i>			
A27*	<i>A. lignieresii</i>	NCTC 4975	Bovine lesion. D1529, J20, SM29
A202*	<i>Actinobacillus</i> sp.	D2146	Ox lymph node, Leicester
A263*	<i>A. lignieresii</i>	NCTC 10563	Bovine tongue lesion. Serotype 1
A44*	<i>A. lignieresii</i>	NCTC 10563	Replicate of A263
A40*	<i>A. lignieresii</i>	NCTC 4191	Bovine lesion
A58	<i>A. equuli</i>	ATCC 13376	Horse joint. Wetmore strain 1706
A283*	<i>Actinobacillus</i> sp.	Frederiksen P598	2008/76
A284*	<i>Actinobacillus</i> sp.	Frederiksen P599	339/77
A285*	<i>Actinobacillus</i> sp.	Frederiksen P600	1004/77
A286*	<i>Actinobacillus</i> sp.	Frederiksen P601	204/78
A28	<i>A. lignieresii</i>	NCTC 4976	Bovine lesions. ATCC 19393, D1530, J21, SM30
A38*	<i>A. lignieresii</i>	NCTC 4976	CEN. Replicate of A28
A59	<i>A. lignieresii</i>	ATCC 13369	Wetmore KC208
A39	<i>A. lignieresii</i>	NCTC 4189	Bovine lesion. TAL, P151
A46	<i>A. lignieresii</i>	NCTC 10565	Sheep lung. Serovar 2
A45	<i>A. lignieresii</i>	NCTC 10564	Bovine tongue. Serovar la
A41*	<i>A. lignieresii</i>	NCTC 4975	Bovine lesions
A43*	<i>A. lignieresii</i>	NCTC 10250	Bovine lymph node. Serovar la
A42*	<i>A. lignieresii</i>	NCTC 4985	Bovine lesions
Phenon 7: <i>Pasteurella haemolytica</i> biovar A			
A88	<i>P. haemolytica</i> A	NCTC 10365	Sheep. J171, SM20, serovar 2
A90	<i>P. haemolytica</i> A	NCTC 10609	Sheep. Serovar 1. ATCC 29696
A102	<i>P. haemolytica</i> A	NCTC 10636	Lamb pneumonia. Serovar 8. ATCC 29699
A91	<i>P. haemolytica</i> A	NCTC 9712	642/56, serovar 2. ATCC 29694
A101	<i>P. haemolytica</i> A	NCTC 10634	Lamb pneumonia. Serovar 7. ATCC 29698
A94	<i>P. haemolytica</i> A	NCTC 10630	Lamb pneumonia. Serovar 5. ATCC 29695
A95	<i>P. haemolytica</i> A	NCTC 10632	Sheep pneumonia. Serovar 6. ATCC 29697
A103	<i>P. haemolytica</i> A	NCTC 10638	CEN. Sheep nose. Serovar 9. ATCC 29700
A106	<i>P. haemolytica</i> A	NCTC 10644	Lamb nose. Serovar 12. ATCC 29702
A207*	<i>P. haemolytica</i> A	LPHL 4585/79	Sheep lung
A104	<i>P. haemolytica</i> T	NCTC 10640	Sheep nose. Serovar 10. ATCC 29706
A135	<i>P. mastitidis</i>	ATCC 10899	Sheep
A96	<i>P. haemolytica</i>	NCTC 9380	TAL, 1266/56, P586, serologically untypable
A137	<i>Pasteurella</i> sp.	D1188	Rabbit snuffles, Leicester University animal house
A223*	<i>P. haemolytica</i>	LPHL 25883/79	Lamb lung
A253*	<i>Pasteurella</i> sp.	LPHL 51807/78	Sheep meningitis
A270*	<i>P. haemolytica</i> A	NCTC 10609	Replicate of A90
A254*	<i>Pasteurella</i> sp.	LPHL 51808/78	Lamb pneumonia
A275*	<i>P. mastitidis</i>	ATCC 10898	Sheep
A97	<i>P. haemolytica</i>	NCTC 10218	T185, serologically untypable
A105	<i>P. haemolytica</i> A	NCTC 10208	Lamb. Serovar 11. ATCC 29701
A160	<i>P. haemolytica</i>	Frederiksen P174	D1902, biovar 3
A161	<i>P. haemolytica</i>	Frederiksen P411	D1903, biovar 3
A126	<i>Pasteurella</i> sp.	LPHL 28552	Source not recorded
Phenon 8: 'pneumotropica-gallinarum' group			
A80*	<i>Pasteurella multocida</i>	NCTC 8282	Dog-bite in man. Parker 36735
A86	<i>P. pneumotropica</i>	NCTC 8141	Mouse lung. TAL, J169, SM18, P421
A140	<i>Pasteurella</i> sp.	D1549	Source not recorded
A141	<i>Pasteurella</i> sp.	D1549	Replicate of A140
A132	<i>P. gallinarum</i>	ATCC 13360	Chicken respiratory tract. NCTC 11187
A133	<i>P. gallinarum</i>	ATCC 13361	CEN. TAL. Chicken sinus. NCTC 11188
A281*	<i>P. gallinarum</i>	ATCC 13361	Replicate of A133
Phenon 9: <i>Pasteurella haemolytica</i> biovar T			
A92	<i>P. haemolytica</i> T	NCTC 10370	Lamb septicaemia. Serovar 3. ATCC 29703
A93	<i>P. haemolytica</i> T	NCTC 10369	Lamb septicaemia. Serovar 4. ATCC 29704
A206*	<i>P. haemolytica</i> T	LPHL 4584/79	Sheep lung
A208*	<i>P. haemolytica</i> T	LPHL 4586/79	Sheep lung

Table 1 (*continued*)

Strain no.	Name as received	Source and collection no.†	Comments‡
A245*	<i>P. haemolytica</i> T	LPHL 51810/78	CEN. Lamb septicaemia
A244*	<i>P. haemolytica</i> T	LPHL 51809/78	Lamb septicaemia
A242*	<i>P. haemolytica</i> T	LPHL 34876/76	Calf lung
A243*	<i>P. haemolytica</i> T	LPHL 34880/76	Calf lung
Phenon 10: <i>Pasteurella multocida</i>			
A72	<i>P. multocida</i>	NCTC 1877	Sheep
A79	<i>P. multocida</i>	NCTC 1737	Pig. Cornelius serological group III
A73	<i>P. multocida</i>	NCTC 5868	Cattle
A74	<i>P. multocida</i>	NCTC 7460	Cow
A76	<i>P. multocida</i>	NCTC 8565	Turkey liver
A78	<i>P. multocida</i>	NCTC 10722	Bovine
A75	<i>P. multocida</i>	NCTC 8281	Dog-bite in man. Parker 36734
A85	<i>P. multocida</i>	NCTC 3195	Bovine. ATCC 19427, J167, SM15
A77	<i>P. multocida</i>	NCTC 10382	Human finger
A113	<i>P. multocida</i>	NCTC 10325	Bovine. Carter serological group D
A112	<i>P. multocida</i>	NCTC 10324	Deer. Carter serological group C
A118	<i>P. multocida</i>	NCTC 1876	Rabbit. Cornelius serological group IV
A114	<i>P. multocida</i>	NCTC 10326	Bovine. Carter serological group E
A117	<i>P. multocida</i>	NCTC 948	Mouse. Cornelius serological group II
A136	<i>P. multocida</i>	NCTC 2771	Cat-bite in man. 21429
A143	<i>Pasteurella</i> sp.	LPHL 37717/74	Brown hopping mouse
A213*	<i>P. multocida</i>	LPHL 9606/79	Cat-scratch in man. Frederiksen var. 5
A107	<i>P. multocida</i>	NCTC 10203	CEN. Bovine. Roberts serovar I
A111	<i>P. multocida</i>	NCTC 10323	Bovine. Carter serological group B
A218*	<i>P. multocida</i>	LPHL 20717/79	Human meningitis
A225*	<i>Pasteurella</i> sp.	LPHL 27397/79	Hedgehog
A269*	<i>P. multocida</i>	NCTC 8565	Replicate of A76
A222*	<i>P. multocida</i>	LPHL 25786/79	Cat-bite in man
A108	<i>P. multocida</i>	NCTC 10201	Avian isolate. Roberts serovar II
A185	<i>P. multocida</i>	LPHL 25034/75	Source not recorded
A186	<i>P. multocida</i>	LPHL 25677/75	Source not recorded
A187	<i>P. multocida</i>	LPHL 27202/75	Source not recorded
A109	<i>P. multocida</i>	NCTC 10202	Deer. Roberts serovar III
A110	<i>P. multocida</i>	NCTC 10322	Pig. TAL, P427. Carter serological group A
A182	<i>P. multocida</i>	D1944	Ferret, Leicester University animal house
A205*	<i>Pasteurella</i> sp.		Ferret nose, Leicester University animal house
A214*	<i>P. multocida</i>	LPHL 11183/79	Human nasal sinus
A115	<i>P. multocida</i>	NCTC 1287	Calf pneumonia. Jones serological group III
A116	<i>P. multocida</i>	NCTC 2417	Rabbit. Cornelius serological group I
A183	<i>P. multocida</i>	Wallace 75/56814	Human abscess from lion mauling. D1943
Phenon 11: <i>Actinobacillus actinomycetemcomitans</i>			
A290*	<i>A. actinomycetemcomitans</i>	NCTC 9709	Human abscess. Opaque colonial variant of A29T
A29T*	<i>A. actinomycetemcomitans</i>	NCTC 9709	CEN. Translucent colonial variant of A290
A30*	<i>A. actinomycetemcomitans</i>	NCTC 9710	Human abscess. TAL
Phenon 12: Satellite			
A163	' <i>Pasteurella anatispestifer</i> '	Eleazer LM	Mallard duck. D1911
Phenon 13: ' <i>Pasteurella bettii</i> '			
A99	' <i>P. bettii</i> '	NCTC 10535	Bartholin cyst. ATCC 23273, CDC 41-5568
A119*	' <i>P. bettii</i> '	NCTC 10534	Urine. ATCC 23275, CDC 41-9471
A120*	' <i>P. bettii</i> '	NCTC 10536	CEN. Infected finger. ATCC 23274, CDC 41-7170
A271*	' <i>P. bettii</i> '	NCTC 10535	Replicate of A99
Phenon 14: ' <i>Actinobacillus seminis</i> '			
A51*	' <i>A. seminis</i> '	NCTC 10851	CEN. Ovine semen, ATCC 15768
A65*	' <i>A. seminis</i> '	ATCC 15768	Same strain as NCTC 10851
A280*	' <i>A. seminis</i> '	NCTC 10851	Replicate of A51
A166*	' <i>A. seminis</i> '	Van Tonder 70-64	D1914
A164*	' <i>A. seminis</i> '	Van Tonder 6201	D1912
Phenon 15: ' <i>Pasteurella haemolytica</i> var. <i>ureae</i> '			
A87	' <i>P. haemolytica</i> var. <i>ureae</i> '	NCTC 10222	Nose of patient with bronchitis. ATCC 29693, J170
A236*	' <i>P. haemolytica</i> var. <i>ureae</i> '	NCTC 10221	CEN. Rhinosinusitis ATCC 29692
A235*	' <i>P. haemolytica</i> var. <i>ureae</i> '	NCTC 10220	Rhinosinusitis. ATCC 29691
A246	<i>P. ureae</i>	LPHL 3901/78	Source not recorded
A234*	' <i>P. haemolytica</i> var. <i>ureae</i> '	NCTC 10219	Ozaena. TAL, ATCC 25976, P161
A273*	' <i>P. haemolytica</i> var. <i>ureae</i> '	NCTC 10222	Replicate of A87
A98*	' <i>P. haemolytica</i> var. <i>ureae</i> '	NCTC 10219	Replicate of A234

Table 1 (continued)

Strain no.	Name as received	Source and collection no.†	Comments‡
Phenon 16: Satellite			
A241*	<i>Pasteurella</i> sp.	LPHL 43451/77	Dog-bite in man
Phenon 17: 'Ross'			
A55	<i>Actinobacillus</i> sp.	NCTC 10801	Sow vagina. Ross 192, ATCC 27072, Frederiksen P624
A68	<i>Actinobacillus</i> sp.	Ross 192	Sow vagina. Same strains as A55
A56*	<i>Actinobacillus</i> sp.	NCTC 10802	CEN. Sow vagina. Ross 63, ATCC 27073, Frederiksen P636
A66*	<i>Actinobacillus</i> sp.	Ross 63	Sow vagina. Same strain as A56
A69*	<i>Actinobacillus</i> sp.	Ross 200	Sow vagina. ATCC 27075
A67	<i>Actinobacillus</i> sp.	Ross 145	Sow vagina. ATCC 27074, Frederiksen P647
A176	<i>Actinobacillus</i> sp.	LPHL 32587/74	Aborted pig foetus stomach
A180*	<i>Actinobacillus</i> sp.	LPHL 48057/75	Sow vagina
A247*	Aerogenic pasteurella	LPHL 43104/76	Aborted pig foetus stomach
A248*	Aerogenic pasteurella	LPHL 46377/76	Aborted pig foetus
A276*	<i>Actinobacillus</i> sp.	Ross 145	Replicate of A67
Phenon 18: Satellite			
A150*	<i>Pasteurella</i> sp.	LPHL 46139	Swine
Phenon 19: Satellite			
A217*	<i>Pasteurella pneumotropica</i>	LPHL 17749/79	Swine
Phenon 20: <i>Pasteurella aerogenes</i>			
A81*	<i>Pasteurella pneumotropica</i>	NCTC 10603	Rabbit lung. Andre strain 9022
A240*	<i>P. pneumotropica</i>	LPHL 48127/78	CEN. Hamster
A272*	<i>P. pneumotropica</i>	Hooper 3	Rat mouth. D1888
A146	<i>P. pneumotropica</i>	Hooper 3	Replicate of A272
A211	<i>Actinobacillus</i> sp.	LPHL 7745/79	Pig liver
A221*	<i>P. aerogenes</i>	ATCC 27883	TAL. Swine intestine
A268*	<i>P. aerogenes</i>	ATCC 27883	Replicate of A221
A224*	<i>Pasteurella</i> sp.	LPHL 27396/79	Rabbit
A203*	<i>Pasteurella</i> sp.	D2147	Rat pneumonia, Leicester University animal house
A238*	<i>P. pneumotropica</i>	LPHL 8832/76	From mouse
A239*	<i>P. pneumotropica</i>	LPHL 50833/77	'Shaws Jird'
A215*	<i>Pasteurella</i> sp.	LPHL 12744/79	Eland calf
A251*	Aerogenic pasteurella	LPHL 49750/77	Mara
Phenon 21: Satellite			
A144	<i>Pasteurella pneumotropica</i>	Hooper 1	Laboratory rodent. D1886
Phenon 22: 'aerogenic group 2'			
A148	<i>Pasteurella</i> sp.	Hooper 5	Guinea pig colitis. D1890
A209*	<i>Pasteurella</i> sp.	LPHL 6052/79	CEN. Man, bite of mara
A210*	<i>Pasteurella</i> sp.	LPHL 6053/79	Man, bite of mara
A249*	Aerogenic pasteurella	LPHL 40026/77	Guinea pig
A250*	Aerogenic pasteurella	LPHL 41889/77	Wallaby
Phenon 23: Satellite			
A252*	<i>Pasteurella</i> sp.	LPHL 51811/78	Dog eye
Phenon 24: Tortoise			
A220*	<i>Pasteurella</i> sp.	LPHL 18329/79	Tortoise
A266*	<i>Pasteurella</i> sp.	LPHL 18329/79	Replicate of A220
Phenon 25: 'ent-b'			
A121	<i>Yersinia enterocolitica</i>	Mollaret 38	Hare. D1703
A122	<i>Y. enterocolitica</i>	Mollaret 225	CEN. Hare. D1708
A181	<i>Y. enterocolitica</i>	NCTC 10460	Chinchilla. Ye91, D1947, 871
A189	<i>Y. enterocolitica</i>	Mollaret Ye198	Chinchilla. Biovar 5
Phenon 26: <i>Yersinia enterocolitica</i>			
A123	<i>Y. enterocolitica</i>	Mollaret 356	Monkey. D1737
A124	<i>Y. enterocolitica</i>	Mollaret 340	Man. D1747
A191	<i>Y. enterocolitica</i>	Mollaret Ye206	Man. Biovar 5
A195	<i>Y. enterocolitica</i>	Mollaret Ye217	Man
A194	<i>Y. enterocolitica</i>	Mollaret Ye216	CEN. Man
A196	<i>Y. enterocolitica</i>	Mollaret Ye241	Man. Biovar 4
A197	<i>Y. enterocolitica</i>	Mollaret Ye244	Man. Biovar 4
A199	<i>Y. enterocolitica</i>	Mollaret Ye250	Man. Biovar 4
A200	<i>Y. enterocolitica</i>	Mollaret Ye252	Man
A171T	<i>Y. enterocolitica</i>	NCTC 10461	Chinchilla. Ye96, P76
A171O	<i>Y. enterocolitica</i>	NCTC 10461	Opaque colonial variant of A171T

Table 1 (*continued*)

Strain no.	Name as received	Source and collection no.†	Comments†
A198	<i>Y. enterocolitica</i>	Mollaret Ye247	Man. Biovar 4
A175	<i>Y. enterocolitica</i>	Mollaret Ye195	Chinchilla
A172	<i>Y. enterocolitica</i>	NCTC 10462	Chinchilla. D1671, Ye92, P72
A173	<i>Y. enterocolitica</i>	NCTC 10463	Chinchilla. D1674, Ye97, P77
A265*	<i>Y. enterocolitica</i>	Mollaret Ye244	Replicate of A197
A125	<i>Y. enterocolitica</i>	LPHL 22790/70	D1752
A193	<i>Y. enterocolitica</i>	Mollaret Ye211	Man. Biovar 5
A201	<i>Y. enterocolitica</i>	Mollaret Ye253	Man
A174	<i>Y. enterocolitica</i>	NCTC 10598	Man. ATCC 23715, Ye636, Billups 1803-68
A190	<i>Y. enterocolitica</i>	Mollaret Ye203	Hare. Biovar 5
Phenon 27: <i>Yersinia pseudotuberculosis</i>			
A127	<i>Y. pseudotuberculosis</i>	Mair 4100	Rabbit. D1535, M39. Serovar IIA
A128	<i>Y. pseudotuberculosis</i>	Mollaret 773	Hare. Serovar IIB, D1536, M43
A129	<i>Y. pseudotuberculosis</i>	Smith 1074	Mink. D1537, M49. Serovar III
A130	<i>Y. pseudotuberculosis</i>	Mair 298	Guinea pig. D1538, M51. Serovar IV
A229*	<i>Y. pseudotuberculosis</i>	Mair 4100	Replicate of A127
A231*	<i>Y. pseudotuberculosis</i>	Smith 1074	CEN. Replicate of A129
A232*	<i>Y. pseudotuberculosis</i>	Mair 298	Replicate of A130
A233*	<i>Y. pseudotuberculosis</i>	Mollaret 75	Guinea pig. Serovar V. D1540, M57
A230*	<i>Y. pseudotuberculosis</i>	Mollaret 773	Replicate of A128
A262*	<i>Y. pseudotuberculosis</i>	Mollaret 773	Replicate of A128
A279*	<i>Yersinia</i> sp.	Holmes 573/74	D1901
Phenon 28: Satellite			
A159	<i>Yersinia</i> sp.	Holmes 573/74	Replicate of A279
Phenon 29: <i>Yersinia pestis</i>			
A138	<i>Y. pestis</i>	D1754	CEN. Nonvirulent strain, M18
A139	<i>Y. pestis</i>	D1754	CEN. Replicate of A138
Phenon 30: 'past-b'			
A89	<i>Pasteurella haemolytica</i> T	NCTC 10371	CEN. Lamb septicaemia. J172
A165	' <i>Actinobacillus seminis</i> '	Van Tonder T981V	D1913
A184	<i>Pasteurella</i> sp.	Jackson CM48770	D1931
Phenon 31: <i>Yersinia philomiragia</i>			
A155	<i>Y. philomiragia</i>	ATCC 25015	TAL. D1897. Moribund muskrat
A156	<i>Y. philomiragia</i>	ATCC 25016	D1898. River water
A157	<i>Y. philomiragia</i>	ATCC 25017	CEN. D1899. River water
A277*	<i>Y. philomiragia</i>	ATCC 25017	Replicate of A157
Phenon 32: <i>Moraxella anatispestifer</i>			
A131*	' <i>Pasteurella anatispestifer</i> '	ATCC 11845	TAL. CEN. Duck septicaemia. NCTC 11014
A282*	' <i>P. anatispestifer</i> '	ATCC 11845	Replicate of A131
A162*	' <i>P. anatispestifer</i> '	Eleazar 1533	Mallard duck. D1910

* Strains thus marked were tested in a second batch of media (see text).

† Sources and abbreviations: CEN, control type of phenon; TAL, type strain in *Approved Lists of Bacterial Names* (Skerman *et al.*, 1980); Mair, Dr N. S. Mair, Leicester University, UK; LPHL, Leicester Public Health Laboratory, UK; Frederiksen, Dr W. Frederiksen, State Serum Institute, Copenhagen, Denmark; Ross, Dr R. F. Ross, Veterinary Medical Research Institute, Iowa State University, Ames, Iowa, USA; Smith, Professor J. E. Smith, University of Surrey, Guildford, UK; NCTC, National Collection of Type Cultures, Colindale, London, UK; ATCC, American Type Culture Collection, Rockville, Md., USA; Holmes, Dr B. Holmes, National Collection of Type Cultures, Colindale, London, UK; D numbers, numbers in the Collection of the Microbiology Department University of Leicester; Cutlip, Dr R. C. Cutlip, USA Department of Agriculture Research Station, Ames, Iowa, USA; Hooper, Dr A. Hooper, Imperial Cancer Research Fund Laboratory, Lincoln's Inn Fields, London WC2A 3PX, UK; J numbers, from Johnson & Sneath (1973); Mollaret, Dr H. H. Mollaret, Institut Pasteur, Paris, France; Van Tonder, Dr E. M. Van Tonder, Regional Veterinary Investigation Centre, Middelburg Cape, South Africa; Eleazar, Dr T. H. Eleazar, Clemson University, Columbia, SC 29202, USA; Wallace, Dr J. Wallace, Public Health Laboratory, Lincoln, UK.

determined by maintaining 10 ml amounts of inoculated BMB at 56 °C for 15 and 30 min; after 24 h incubation at 37 °C the cultures were plated onto blood agar and examined for growth after 24 h.

Growth on special media and inhibitors. Tolerance of inhibitory compounds was tested in Replidishes. Visible growth on BMA containing 3, 4, 5, 6, 7 and 8% (w/v) NaCl was recorded after 48 h. Visible growth was recorded after 48 h on BMA containing 0.1% and 1% (v/v) Teepol L (BDH); 0.75% (w/v) sodium arsenate; 9 p.p.m. (w/v) acriflavine; 10 p.p.m. (w/v) 4,4'-diamidinodiphenylamine; 0.3% (w/v) boric acid; 1% (v/v) tributyrin; 200 and 30

p.p.m malachite green; 0.01, 0.02 and 0.05% (w/v) sodium azide; 0.07% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 10 p.p.m (w/v) brilliant green; 2 p.p.m (w/v) crystal violet; and 0.05% (w/v) potassium tellurite. Reduction of tellurite was recorded as blackening. Growth in BMB containing 0.1% (w/v) potassium tetrathionate, and reduction of tetrathionate (yellow colour) were recorded after 3 d. Visible growth was recorded after 48 h on MacConkey agar (Oxoid), deoxycholate-citrate agar (Oxoid), and TCBS agar (Oxoid). Growth on violet red bile agar (Oxoid) and precipitation of bile salts were recorded after 48 h.

Reduction of tetrazolium was tested by incorporating 0.1% and 0.01% (w/v) 2,3,5-triphenyltetrazolium chloride and 1% (w/v) glucose in BMA adjusted to pH 6.0. The inoculated plates were incubated for 48 h aerobically or anaerobically (GasPak, Becton-Dickinson). Red or white colonies were noted and reduction was scored if the surrounding medium also became red. Growth in BMB containing methylene blue 200 p.p.m or janus green 80 p.p.m was recorded 48 h after inoculation. Reduction of the dyes and gas production were also noted. Acidity or alkalinity on BMA containing bromothymol blue and buffered to pH 7.0 (green) was recorded at 48 h.

Biochemical tests. The methyl-red and Voges-Proskauer tests were done at 25 and 35 °C after 7 d on cultures of BMB containing 0.5% (w/v) K_2HPO_4 and 0.5% (w/v) glucose. For the Voges-Proskauer test an equal amount of 40% (w/v) KOH and a few drops of 1% (w/v) α -naphthol were added, and a red colour recorded as positive. The Voges-Proskauer test was also recorded after 48 h in the API50E galleries according to the manufacturer's instructions. Catalase was detected by emulsifying 24 h colonies from BMA in 3% (v/v) H_2O_2 and recording bubbles. Oxidase was tested by the method of Kovacs (1956) on growth from 24 h cultures on BMA and a purple colour within 20 s was scored as positive. Indole production was tested after 48 h in BMB by the method of Cowan (1974). Production of H_2S from BMB after 2 d was detected by lead acetate paper, and H_2S in triple sugar iron agar (Oxoid) after 7 d. Nitrate reduction was tested after 7 d by the method of Cowan (1974) on BMB containing 0.1% (w/v) KNO_3 and Durham tubes were included to test for gas production: destruction of nitrite was tested by the same method on BMB containing 0.01 and 0.001% (w/v) NaNO_2 .

Gelatin liquefaction after 5 d was determined by inoculation of BMB containing 12% (w/v) gelatin and cooling to 4 °C before reading. Tributyrin hydrolysis was recorded as clear zones on BMA containing 1% (v/v) tributyrin after 48 h. Hydrolysis of casein was detected by the method of Cowan (1974) by streaking on agar plates composed of one part of skim milk medium (Difco) with one part of double strength BMA, and examining for clearing after 48 h. Hydrolysis of Tweens 20, 40, 60 and 80 was tested as clearing at 48 h on plates containing 1% (v/v) Tween. Digestion of tyrosine (0.5%, w/v) in BMA was detected as a turbid zone after 48 h.

Urease was determined on the medium of Cowan (1974) after 2 d, and also recorded in the API50E galleries after 24 h. Phosphatase was determined by the method of Cowan (1974) after 7 d incubation on BMA containing 0.01% (w/v) phenolphthalein diphosphate sodium salt as a red colour on flooding with strong ammonia solution. Lecithinase was determined after 48 h as turbidity in plates of BMA containing egg yolk emulsion (Oxoid SR 47; 1%, v/v, added after cooling to 55 °C), after flooding with 1% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Deoxyribonuclease was detected on DNAase agar (Oxoid CM321) by flooding with 1 M-HCl after 5 d incubation and examining for clearing; orange and brown fluorescence under UV light (366 nm) was also recorded. Aesculin hydrolysis was determined in the API galleries after 24 h.

Lysine and ornithine decarboxylases, arginine dihydrolase and tryptophan deaminase were recorded in API50E galleries after 24 h according to the manufacturer's instructions. Citrate utilization was determined by the method of Christensen as modified by Cowan (1974) as growth at 48 h, and also in the API50E galleries (see below) according to the manufacturer's instructions. *o*-Nitrophenyl β -D-galactopyranoside (ONPG) hydrolysis was recorded in the galleries after 24 h. Production of levan as mucoid growth, and acidity on BMA containing 5% (w/v) sucrose and 30 p.p.m bromothymol blue were determined after 2 d incubation. Gas from glucose was recorded at 48 h in BMB containing 1% (w/v) glucose and a Durham tube and in the medium of Hugh & Leifson (1953). Fermentative reaction and alkalinity were also determined in the latter medium after 48 h: decolorization of the indicator at 48 h was also recorded.

Production of acid from carbohydrates, etc. Acid production from carbohydrates was determined in API50E galleries (phenol red as indicator) according to the manufacturer's instructions: growth from overnight cultures on BMA was suspended in API50E medium to give the turbidity of McFarland standard 2; cupules were inoculated and acidity was recorded at 24 h.

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

The $t \times n$ matrix consisted of strains scored for 155 characters, after excluding eight invariant characters (see Table 3). Most characters were coded 0 for negative and 1 for positive. The characters from the API kits were coded from 0 for negative to 5 for a strong positive reaction. The similarity coefficients used were the Gower coefficient (S_G), the Simple Matching coefficient (S_{SM}) and the Pattern Difference (D_P). Single Linkage and average linkage (UPGMA) cluster analyses were done and the vigour of each strain was 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 API50E results 0–2 were scored negative and 3–5 positive.

Test reproducibility was estimated by limited replication of the tests, and by duplication of some strains. These duplicate strains were carried through as independent strains, and their identities are shown in Table 1. Two strains (A29, A171) showed pronounced colony variants, which were purified and carried through the study separately. Test error was calculated by formulae 1 and 4 in Sneath & Johnson (1972). It was found necessary to do this study in two separate stages, whereby about half the strains were tested on the first occasion, and the remainder on a second occasion with a different batch of culture media. The strains examined on the second occasion, therefore, are marked with asterisks in Table 1.

Other calculations. The overlap statistics of the phenons were calculated by the computer program OVCLUST (Sneath, 1979*a*). In this, the number of operational taxonomic units (OTUs) in each phenon, and the inter- and intra-group distances d [based on the relation $d = \sqrt{1 - S_{SM}}$] are used to calculate an index of overlap, V_G . This overlap can then be tested for significance against a chosen critical level of overlap, V_{crit} , by a non-central t -test. The test is not available if either phenon contains only one OTU.

The program DIACHAR (Sneath, 1980*a*) was used to list the most diagnostic characters of phenons, and the program CHARSEP was used to calculate VSP separation indices (Sneath, 1979*b*) so as to list the characters in order of their diagnostic value. The program MOSTTYP (Sneath, 1980*b*) was used to confirm that good identifications could be made from an identification matrix of phenon percentage properties, and the program OVERMAT (Sneath, 1980*c*) was used to check overlap in this, by the same statistics as OVCLUST.

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. In order to position the satellite strains (singletons), these were included as phenons of one OTU.

The amount of distortion between the similarities in the similarity matrix and the corresponding similarities implied by the phenogram was assessed by the cophenetic discrepancy Δ_j as calculated by Sneath *et al.* (1981).

RESULTS

Test reproducibility

There were four classes of duplicated strains: (a) nine replicates on the same batch of medium; (b) 20 replicates where one was tested on the first batch of medium and the other on the second batch; (c) two cases where two colony variants were tested on the same batch of medium; and (d) six instances where the same isolate was received from different donors and tested on the same batch of medium. The mean percentage S_{SM} values of the duplicated pairs, with standard deviations, were (a) 90.3 ± 4.0 ; (b) 87.7 ± 3.2 ; (c) 96.0 ± 0.3 ; (d) 93.1 ± 1.2 . The test error p is best estimated from (a) and (b), and this gives a rather high value for p of about 6.1%.

The mean difference in vigour between replicates of all the four classes (a) to (d) was much the same, averaging about 0.03. The two batches of medium did not differ significantly in the ease with which tests became positive, as judged by cases where one replicate was on one batch and the other on the other batch of medium (difference in means 0.008).

The batch of medium had no pronounced effect on the position of strains within the clusters. There was a noteworthy effect only in phenon 27, where strains tested in the second batch (including some replicates) are in the lower half of the phenon (Table 1).

Main cluster analysis

The UPGMA analysis with S_G (Fig. 1) is used as the basis for the taxonomic description. Twenty-four groups of strains were considered reasonably distinct, and in addition there were eight satellites, which were treated as single-membered phenons. Phenons 24 and 29, though based on replicates of single strains, have been retained formally as separate phenons, as they give an indication of the variability due to the lack of complete test reproducibility. These are numbered as phenons 1–32 in Table 1. There were three main areas: A, the actinobacilli and pasteurellas (phenons 1–24); B, the yersinias (phenons 25–29); and C, a miscellaneous group (phenons 30–32).

Description of phenons

Constituent strains of phenons are shown in Table 1. The parameters of the phenons, with intercentroid distances, are shown in Table 2. The percentages of positive test reactions are given in Table 3.

Fig. 1. Simplified UPGMA phenogram based on S_G . The cophenetic correlation was 0.823.

Area A: actinobacilli and pasteurellae (phenons 1–24). Phenon 1 is *Actinobacillus suis*, though the type strain CCM 5586 was not available. We have since obtained it and found its reactions to agree with the majority reactions of strains in phenon 1, except that CCM 5586 did not acidify D(–)-arabinose. This phenon is characterized by positive results for β haemolysis, lecithinase, and acidity from salicin, raffinose, melibiose and cellobiose. Tween 60 is usually hydrolysed.

Phenon 2 is *Actinobacillus equuli*, characterized particularly by positive results for raffinose, lactose and trehalose, and sensitivity to crystal violet. Phenon 3, a satellite strain from a monkey, is notable for clearing on tyrosine agar, and acid from amygdalin and glycogen.

Phenon 4. This group of strains, 'BLG', from bovine lymphangitis in India (Jayaraman & Sethumadavan, 1974), forms a compact group. They are notable in not reducing nitrate, and in being sensitive to azide and positive for arbutin but negative for oxidase.

Table 2. *Parameters of phenons and intracentroid distances*

The values are from data converted to positive and negative scores (see Methods). The entries in the body of the table are the intercentroid distances, $d = \sqrt{(1 - S_{SM})}$, from which Fig. 2 was derived. The mean vigour of each phenon is the mean proportion of characters with positive states, and SD is the standard deviation of the vigour of the strains. The mean distance of strains of a phenon to their own centroid is $\bar{d}_J$, 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 phenon J , and t_J is the number of strains in J . It can be shown (Sneath, 1974) that $\bar{d}_J$ is $\sqrt{\frac{1}{2}(1 - \bar{S}_{SM})}$, where $\bar{S}_{SM}$ is the mean intraphenon S_{SM} obtained as the mean of the $t_J(t_J - 1)/2$ intraphenon S_{SM} values. NA, Not applicable.

Phenon	1	2	3	4	5	6	7	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
No. of strains	38	13	1	3	10	6	7	8	9	10	11	12	13	14	15																	
Mean vigour	0.451	0.384	0.438	0.332	0.350	0.340	0.342	0.365	0.292	0.323	0.235	0.246	0.230	0.182	0.235																	
SD	0.021	0.015	NA	0.032	0.021	0.029	0.037	0.029	0.023	0.026	0.009	NA	0.022	0.035	0.023																	
$\bar{d}_J$	0.225	0.240	NA	0.235	0.253	0.265	0.263	0.293	0.234	0.267	0.176	NA	0.244	0.236	0.234																	

Phenon 5 is referred to as the 'Mair' group. These strains were isolated from swine in Leicestershire, UK, and given by Dr N. S. Mair. They were associated with abortions in sows, but may occur in other animals, because A.212, although rather peripheral, came from a marmot. They show a characteristic orange fluorescence on DNAase agar. Some other phenons showed a brown fluorescence (Table 3) but it was not very strong. The phenon is also characterized by being sensitive to malachite green and 1% Teepol, and is usually positive for ornithine decarboxylase.

Phenon 6 is *Actinobacillus lignieresii*, and diagnostic properties include reduction of nitrite, acid from mannitol, D(+)-xylose and sucrose, but not from L(+)-arabinose and trehalose.

Phenon 7 contains the strains of *Pasteurella haemolytica* biovar A, though strain A104 is recorded as biovar T (see Discussion). We found, as did Sakazaki *et al.* (1981), that they do not always acidify L-arabinose promptly. These strains are characterized by acidifying sorbitol and mannitol but not trehalose; they are sensitive to crystal violet.

Phenon 8 contains the type strains of both *Pasteurella pneumotropica* and *Pasteurella gallinarum*, and is referred to as the 'pneumotropica-gallinarum' group (for the distinction between these species, see Discussion). They are trehalose positive and mannitol negative, and grow on violet red bile agar and on 3% NaCl.

Phenon 9 consists exclusively of strains of *P. haemolytica* biovar T. They are characteristically catalase negative, sorbitol and trehalose positive but galactose negative and urease negative. They often showed delayed fermentation in Hugh & Leifson medium.

Phenon 10. This large phenon corresponds to *Pasteurella multocida*. Characteristically, the strains are urease and ONPG negative, sensitive to 1% Teepol, but grow on 3% NaCl and are usually sucrose and mannose positive.

[illegible]

Phenon 17 is referred to as the 'Ross' group. It contains strains from the vagina of postparturient sows (Ross *et al.*, 1972), and some similar strains isolated in England from aborted piglets in 1974-1976. The phenon is, however, quite distinct from *A. suis* or the 'Mair' group. It is characteristically inositol and sorbitol positive but sucrose negative. Janus green is reduced.

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

		NT, Not tested.										
No.	Character	Phenon . . .	1	2	3	4	5	6	7	8	9	10
<i>Colonial and morphological features</i>												
Constant characters: Gram-negative, greyish semitransparent colonies (some variants were more opaque, see Table 1)												
1.	Rods numerous		100	100	100	100	100	63	100	100	88	63
2.	Coccobacilli numerous		51	85	100	33	60	84	73	43	88	82
3.	Cocci numerous		13	8	0	0	0	32	13	13	13	36
4.	Length <1 µm		94	100	100	100	100	73	100	71	100	91
5.	Length 1-2 µm		100	92	100	100	89	42	77	86	50	40
6.	Length >2 µm		17	8	0	33	10	16	0	28	0	9
7.	Width <0.25 µm		3	8	0	0	10	0	5	28	0	9
8.	Width 0.25-0.5 µm		82	100	100	33	100	100	96	100	100	97
9.	Width 0.5-1.0 µm		49	69	100	67	30	16	50	0	38	13
10.	Straight-sided rods		97	100	100	100	89	91	95	100	100	100
11.	Organisms predominantly single		82	92	100	100	70	63	55	71	63	60
12.	Filaments present		97	92	0	100	60	32	36	28	38	20
13.	Stain strongly with Gram counterstain		66	31	0	100	80	63	86	86	88	54
14.	Bipolar stain marked		77	77	100	33	20	21	36	13	25	26
15.	Extracellular slime in Gram stain		20	69	0	0	0	11	59	57	38	3
16.	Motile 35 °C, hanging drop		0	0	0	0	0	5	0	0	0	0
17.	Motile 35 °C, semisolid agar		NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
18.	Motile 25 °C, semisolid agar		NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
19.	Greening of sheep blood, 24 h		0	23	0	0	10	16	4	0	13	6
20.	Greening of sheep blood, 48 h		0	31	0	33	10	32	9	28	13	11
21.	Haemolysis of sheep blood, 24 h		100	0	0	0	40	21	70	0	38	0
22.	Haemolysis of sheep blood, 48 h		100	0	0	0	70	21	70	0	50	3
<i>Growth temperature and heat resistance</i>												
Constant characters: all grew at 37 °C; none required added CO ₂ for reasonable growth.												
23.	Growth at 4 °C		0	0	0	0	0	0	0	0	0	0
24.	Growth at 10 °C		0	0	100	0	0	0	0	0	0	0
25.	Growth at 15 °C		8	0	100	33	0	0	4	0	13	28
26.	Growth at 45 °C		0	0	0	0	0	0	0	0	0	0
27.	Resistance to 56 °C, 15 min		0	0	100	0	0	0	0	0	0	3
28.	Resistance to 56 °C, 30 min		0	0	0	0	0	0	0	0	0	0
<i>Growth on special media and inhibitors</i>												
Constant characters: no white colonies on 0.1% tetrazolium anaerobically; all grew but gave no gas in methylene blue medium												
29.	Growth on 3% NaCl		89	69	100	0	10	36	44	100	0	97
30.	Growth on 4% NaCl		8	0	100	0	0	5	0	0	0	57
31.	Growth on 5% NaCl		3	0	0	0	0	0	0	0	0	3
32.	Growth on 6% NaCl		0	0	0	0	0	0	0	0	0	3
33.	Growth on 7% NaCl		0	0	0	0	0	0	0	0	0	3
34.	Growth on 8% NaCl		0	0	0	0	0	0	0	0	0	0
35.	Growth on Teepol L, 0.1%		100	100	100	100	100	95	96	100	100	91
36.	Growth on Teepol L, 1.0%		63	0	0	0	0	53	13	13	63	3
37.	Growth on arsenate		21	8	0	0	0	18	9	13	0	21
38.	Growth on acriflavine		0	0	0	100	0	0	13	13	0	3
39.	Growth on 4,4'-diamidinodiphenylamine		80	69	100	100	10	16	4	33	0	94
40.	Growth on boric acid		24	46	0	33	20	32	21	57	38	86
41.	Growth on tributyrin		100	100	100	100	100	100	100	100	100	100
42.	Growth on malachite green, 30 ppm		100	69	0	100	0	89	75	71	100	40
43.	Growth on malachite green, 200 ppm		3	0	0	0	0	16	4	13	0	3
44.	Growth on sodium azide, 0.01%		100	100	100	0	100	100	100	86	100	94
45.	Growth on sodium azide, 0.02%		100	85	0	33	80	79	67	43	13	43
46.	Growth on sodium azide, 0.05%		26	8	0	0	0	11	4	13	0	9
47.	Growth on copper sulphate		24	15	100	33	0	11	0	13	0	0
48.	Growth on brilliant green		40	8	100	33	0	32	4	57	13	11
49.	Growth on crystal violet, 2 ppm		66	0	100	100	10	53	4	71	50	80
50.	Growth on tellurite		0	0	0	33	10	0	0	28	0	3
51.	Reduction of tellurite		73	23	100	67	50	16	67	71	25	51

Table 3 (continued)

11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
100	100	100	80	100	0	100	100	100	100	100	40	100	0	75	100	82	100	NT	100	NT	100
100	100	100	60	86	100	0	0	0	77	100	100	0	100	100	95	91	100	NT	0	NT	67
100	0	0	0	0	0	0	0	0	0	0	40	0	NT	0	5	18	0	NT	0	0	0
100	100	100	80	100	100	82	100	100	100	100	100	100	0	100	100	100	100	NT	50	NT	100
0	0	0	80	86	0	100	100	100	39	0	0	100	0	25	48	55	0	NT	100	0	33
0	0	0	80	43	0	73	0	0	8	0	0	100	0	0	5	27	0	NT	100	0	33
67	100	50	0	13	0	9	100	100	23	0	20	0	0	0	0	0	0	NT	0	0	67
33	100	50	100	57	100	91	0	0	85	100	80	100	100	75	95	91	100	NT	100	NT	67
0	0	0	40	43	0	46	0	0	0	0	0	0	0	75	43	73	100	NT	50	0	0
100	100	100	100	86	NT	91	100	100	100	100	NT	100	NT	33	95	89	100	NT	50	NT	67
33	100	50	20	86	0	73	100	100	92	100	40	0	100	100	38	82	0	NT	50	NT	33
100	100	25	80	71	0	91	0	0	46	0	100	100	0	0	52	64	100	NT	50	0	100
33	100	0	60	57	0	46	0	0	54	0	40	0	0	100	100	100	100	NT	100	0	33
0	0	0	60	57	0	46	0	0	15	0	0	0	0	0	0	0	100	NT	0	0	0
0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	NT	0	100	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	NT	33	0	0
NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0	NT	NT	25	20	0	0	NT	0	0	NT
NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0	NT	NT	100	85	27	0	NT	0	0	NT
0	0	25	20	28	100	0	0	0	31	0	0	0	50	0	0	46	0	NT	0	0	67
0	0	50	60	57	100	0	0	0	62	0	0	100	50	0	5	64	0	NT	0	25	67
0	0	0	0	0	0	73	0	0	8	0	0	0	0	0	0	0	0	NT	0	0	0
0	0	0	0	0	0	73	0	0	8	0	0	0	0	0	0	0	0	NT	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	82	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	100	NT	67	25	0
0	0	25	0	0	0	0	0	0	8	0	0	0	100	100	100	100	100	NT	100	100	0
0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	10	9	0	0	0	0	0
0	NT	0	0	0	0	0	0	0	0	0	0	0	0	0	40	0	0	0	33	0	0
0	NT	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	33	0	0
0	0	25	40	57	100	64	0	0	92	0	100	0	100	50	100	100	100	NT	100	100	100
0	0	0	20	0	0	0	0	0	23	0	100	0	100	50	95	91	100	NT	67	100	33
0	0	0	0	0	0	0	0	0	8	0	0	0	100	50	95	27	100	0	67	100	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	95	18	100	0	100	100	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	10	100	0	100	100	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	9	100	0	67	100	0
33	0	100	80	43	100	100	100	100	100	100	100	100	100	100	100	100	100	100	67	100	0
0	0	0	0	0	0	73	0	100	85	100	100	0	100	100	100	100	100	100	67	100	0
0	0	0	0	0	0	0	0	0	46	0	80	0	100	0	57	55	NT	0	67	25	67
0	0	0	0	0	0	27	0	0	46	0	100	0	0	100	100	100	100	100	33	0	0
0	0	0	0	0	100	80	0	0	100	0	100	100	100	50	100	100	100	100	0	25	0
0	0	75	60	0	100	73	100	100	92	100	100	100	100	75	100	100	NT	100	33	25	67
67	0	75	100	100	100	91	100	100	100	100	100	100	100	100	33	100	NT	100	100	50	33
67	100	25	40	0	0	55	100	100	100	100	100	100	100	100	100	100	100	100	100	25	0
0	0	0	0	0	0	0	0	0	23	100	100	0	0	100	0	100	100	100	33	0	33
0	100	100	100	100	0	91	100	100	92	100	100	100	100	100	100	91	0	100	67	100	0
0	0	100	80	86	0	36	0	100	77	0	100	0	100	50	52	91	0	100	67	50	0
0	0	25	20	13	0	0	0	0	8	0	80	0	100	0	38	91	0	0	0	25	0
0	0	0	0	0	0	0	100	0	46	0	20	0	0	100	100	100	100	100	100	0	0
0	0	25	0	0	0	9	0	0	62	100	100	NT	0	100	100	91	100	100	67	0	0
100	0	75	0	0	0	55	100	100	100	100	100	100	100	100	100	100	100	100	33	0	0
0	100	0	0	0	0	0	0	0	23	0	0	0	0	0	5	100	100	100	0	0	0
33	100	25	0	13	0	18	0	0	39	100	100	100	0	75	100	100	100	100	100	25	0

Table 3 (continued)

No.	Character	Phenon . . .	1	2	3	4	5	6	7	8	9	10
52.	Growth on tetrathionate		100	100	100	100	100	100	100	100	75	100
53.	Reduction of tetrathionate		79	92	0	100	100	89	100	86	100	100
54.	Growth on MacConkey agar		100	100	100	67	70	100	96	100	100	100
55.	Growth on deoxycholate-citrate agar		0	0	0	0	0	0	0	17	0	3
56.	Growth on TCBS agar		0	0	100	0	0	0	0	0	0	0
57.	Growth on violet red bile agar		100	100	100	100	NT	95	96	100	100	28
58.	Precipitation on violet red bile agar		100	100	0	0	20	89	64	43	0	0
59.	White colonies on 0.01% tetrazolium, aerobic		26	50	NT	67	0	5	35	13	0	NT
60.	Red colonies on 0.01% tetrazolium, aerobic		66	50	NT	0	67	95	50	13	13	50
61.	Reduction on 0.01% tetrazolium, aerobic		100	100	NT	100	100	100	100	100	100	NT
62.	White colonies on 0.1% tetrazolium, aerobic		0	NT	NT	67	NT	NT	NT	NT	NT	NT
63.	Red colonies on 0.1% tetrazolium, aerobic		0	NT	NT	0	NT	NT	NT	NT	NT	NT
64.	Reduction on 0.1% tetrazolium, aerobic		100	NT	NT	100	NT	NT	NT	NT	NT	NT
65.	White colonies on 0.01% tetrazolium, anaerobic		0	0	0	0	0	0	0	0	0	0
66.	Red colonies on 0.01% tetrazolium, anaerobic		100	92	100	33	100	84	57	71	100	100
67.	Reduction on 0.01% tetrazolium, anaerobic		100	100	100	100	100	100	100	100	100	100
68.	Red colonies on 0.1% tetrazolium, anaerobic		40	0	NT	NT	100	NT	39	NT	NT	NT
69.	Reduction on 0.1% tetrazolium, anaerobic		100	100	NT	100	100	100	89	89	NT	100
70.	Reduction of methylene blue		32	39	100	67	50	63	75	28	13	20
71.	Growth on janus green		100	100	100	100	100	100	100	100	100	100
72.	Reduction of janus green		0	0	0	0	0	0	0	0	0	0
73.	Gas in janus green		3	0	0	0	0	0	0	0	0	0
74.	Acid on BMA		92	77	0	100	44	89	71	71	38	11
75.	Alkali on BMA		0	0	0	0	22	0	0	13	0	23
<i>Biochemical properties</i>												
Constant characters: none were oxidative in Hugh & Leifson medium												
76.	Methyl-red positive 35 °C		34	69	100	100	80	36	0	43	13	86
77.	Methyl-red positive 25 °C		34	69	0	100	70	68	4	13	0	54
78.	Voges-Proskauer 35 °C		8	39	0	0	50	21	63	100	75	54
79.	Voges-Proskauer 25 °C		3	8	100	0	0	21	4	13	0	43
80.	Voges-Proskauer 37 °C (API)		3	8	100	0	0	0	0	43	0	23
81.	Catalase		95	46	100	100	89	57	96	86	0	100
82.	Oxidase		100	100	0	0	100	84	100	86	100	94
83.	Indole		0	0	0	0	0	0	0	28	0	72
84.	H ₂ S from broth		0	0	0	0	40	16	4	0	0	6
85.	H ₂ S in triple sugar iron agar		0	0	0	0	0	0	0	0	0	0
86.	Nitrate reduction		100	100	100	0	100	100	100	100	100	100
87.	Gas from nitrate		5	8	0	0	0	11	0	0	0	0
88.	Destruction of 0.001% nitrite		100	100	100	0	89	100	82	100	63	69
89.	Destruction of 0.01% nitrite		28	62	100	0	89	100	38	86	0	23
90.	Gelatin liquefaction		0	0	0	0	0	0	0	0	0	0
91.	Tributyrin hydrolysis		0	0	0	0	0	0	0	0	0	0
92.	Casein hydrolysis		0	0	0	0	0	0	NT	0	0	0
93.	Hydrolysis of Tween 20		3	0	0	0	0	0	0	0	0	0
94.	Hydrolysis of Tween 40		95	92	NT	33	0	6	0	0	0	48
95.	Hydrolysis of Tween 60		100	92	100	0	0	6	4	0	0	66
96.	Hydrolysis of Tween 80		28	0	0	0	0	0	0	0	0	0
97.	Tyrosine digestion		0	0	100	0	0	0	0	0	0	34
98.	Urease		100	92	100	100	100	95	0	56	0	0
99.	Urease (API)		89	62	100	100	89	63	4	43	0	3
100.	Phosphatase		100	100	0	100	100	100	100	100	100	100
101.	Lecithinase		100	62	0	0	0	0	0	0	0	0
102.	Deoxyribonuclease		0	0	0	0	0	5	0	0	0	0
103.	Orange fluorescence on DNAase agar		0	0	0	0	100	0	0	0	0	0
104.	Brown fluorescence on DNAase agar		0	0	0	0	0	11	38	71	63	66
105.	Aesculin hydrolysis (API)		100	0	100	100	30	0	21	0	75	6
106.	Arginine dihydrolase		3	0	0	0	0	0	4	0	0	3
107.	Lysine decarboxylase		0	8	0	0	0	0	0	0	0	0
108.	Ornithine decarboxylase		0	0	0	0	89	0	0	43	0	3
109.	Tryptophan deaminase		0	0	0	0	0	0	0	0	0	0
110.	Citrate utilization (Christensen)		0	0	0	0	0	0	0	0	0	0
111.	Citrate utilization (API)		0	8	0	0	10	0	0	13	0	3

Table 3 (continued)

11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
100	100	25	60	13	0	100	0	100	100	100	100	100	100	100	100	100	100	NT	100	75	33
100	100	NT	NT	NT	NT	100	NT	100	92	0	60	0	50	0	5	0	0	NT	100	100	NT
33	0	75	0	43	0	82	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0
0	0	0	0	0	0	0	0	0	15	0	20	0	100	100	100	100	100	100	33	0	0
0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	52	0	0	NT	0	25	0
0	0	75	0	0	0	82	0	0	100	100	100	0	100	100	100	100	100	100	67	0	0
NT	NT	0	NT	NT	NT	89	NT	NT	77	100	80	NT	100	0	10	0	0	0	0	0	NT
0	NT	NT	NT	NT	NT	25	NT	0	0	0	0	0	100	25	95	100	100	100	0	0	0
0	NT	NT	NT	NT	NT	63	NT	100	92	100	80	0	0	75	5	0	0	0	100	100	100
0	NT	NT	NT	NT	NT	100	NT	100	100	100	100	100	100	100	24	0	0	0	100	100	100
NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0	NT	NT	0	10	64	0	100	0	NT	NT
NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	100	NT	NT	100	86	0	0	0	100	NT	NT
NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	100	NT	NT	100	100	100	0	0	100	NT	NT
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43	100	100	100	0	0	0
100	100	100	100	82	100	91	100	100	85	100	100	100	100	100	57	0	0	0	100	67	100
100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	13	0	0	0	100	67	100
NT	100	NT	NT	NT	NT	NT	NT	NT	100	100	100	NT	100	100	100	100	100	100	100	NT	NT
NT	100	NT	NT	NT	NT	NT	NT	NT	100	100	100	NT	100	100	100	100	100	100	100	NT	NT
0	0	75	0	13	0	64	0	100	39	100	100	0	100	100	95	91	100	NT	100	25	0
100	100	100	100	0	0	100	100	100	100	100	100	100	100	100	100	100	100	NT	100	75	0
0	0	100	0	NT	NT	100	100	100	31	100	60	100	0	0	19	0	0	NT	0	0	NT
0	0	0	0	NT	NT	0	0	0	0	0	60	0	0	0	19	0	0	NT	0	0	NT
67	NT	75	25	28	100	67	100	0	8	100	0	100	0	0	62	27	NT	0	0	0	0
0	NT	0	0	0	0	0	0	0	17	0	20	0	50	0	71	46	NT	100	100	25	50

100	0	100	0	13	0	46	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0
100	0	100	20	82	0	9	100	100	92	100	100	100	100	100	95	100	100	NT	0	0	0
0	0	0	0	13	100	0	0	0	69	0	0	0	NT	0	24	0	0	0	0	0	67
0	0	0	0	0	0	0	0	0	39	0	0	100	50	0	80	0	0	NT	0	0	0
0	0	25	0	0	0	0	0	0	8	0	20	NT	0	50	10	0	0	0	0	0	0
100	100	50	100	57	100	100	100	100	92	0	100	0	100	100	100	100	100	100	67	100	67
NT	100	0	60	86	100	91	0	100	46	0	100	100	100	50	0	0	0	0	67	100	100
0	0	0	0	0	0	0	100	100	39	0	0	NT	0	0	24	0	0	0	0	0	0
0	0	0	0	0	0	100	0	0	77	0	80	100	100	0	0	0	0	NT	0	25	0
0	0	0	0	0	0	64	0	0	0	0	0	0	0	0	0	0	0	NT	0	100	0
100	NT	100	100	100	100	91	100	100	100	100	100	100	100	25	100	100	100	100	67	0	0
0	NT	0	0	13	0	0	0	0	15	0	80	0	0	0	0	0	0	0	0	0	0
67	0	75	50	100	0	100	100	100	100	100	100	100	100	100	100	36	0	0	0	0	100
0	0	75	0	0	0	73	100	100	77	100	100	0	0	100	95	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
0	NT	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	NT	0	0	NT	NT
NT	NT	NT	NT	NT	NT	0	NT	NT	0	0	20	NT	0	0	0	0	0	0	0	0	NT
NT	100	0	0	13	0	0	0	0	0	0	0	0	0	0	5	55	0	0	0	100	100
NT	100	0	NT	80	NT	0	0	0	0	0	0	0	0	0	5	9	0	0	0	100	100
NT	100	0	NT	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	100	67
0	NT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	100	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NT	0	0	0	0
0	100	0	0	100	100	100	100	100	92	100	100	0	0	100	100	100	100	0	100	0	67
0	0	0	0	13	0	55	100	0	54	0	0	0	0	0	100	100	91	100	0	33	0
100	100	100	0	100	100	100	0	100	92	0	100	100	100	0	10	18	0	0	33	100	100
0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	NT	0	0	25	33
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	100	0	0	0	0	0	0	0	46	0	0	0	0	0	0	18	100	100	0	0	67
0	0	0	40	0	0	64	0	0	8	0	0	0	100	0	10	100	100	100	33	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	40	0	0	0	0	0	23	100	0	0	0	50	100	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	NT	0	0	0	0	0	0	33	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	24	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	67	0	33

Table 3 (*continued*)

No.	Character	Phenon . . .	1	2	3	4	5	6	7	8	9	10
112.	ONPG		87	69	100	0	30	79	57	43	0	0
113.	Levan production		0	8	0	0	0	0	4	0	63	0
114.	Acid on 5% sucrose agar		100	100	100	0	100	100	100	100	100	97
115.	Gas from glucose broth		0	0	0	0	0	0	0	0	NT	0
116.	Gas in Hugh & Leifson medium		0	0	0	0	10	0	0	0	0	0
117.	Fermentative reaction, Hugh & Leifson medium		100	100	100	100	100	100	100	100	100	13
118.	Alkalinity, Hugh & Leifson medium		0	0	0	0	0	0	0	0	0	0
119.	Decolorization, Hugh & Leifson medium		0	0	0	0	10	0	0	0	0	0
<i>Acid from carbohydrates, etc.</i>												
Constant characters: all strains were negative with amylose and methyl-D-xyloside												
120.	Adonitol		0	0	0	0	0	0	0	0	0	0
121.	Amygdalin		32	0	100	0	0	0	17	0	25	3
122.	D(-)-Arabinose		61	15	0	0	30	42	57	86	0	36
123.	L(+)-Arabinose		87	8	100	100	100	0	NT	28	0	23
124.	Arbutin		97	0	100	100	0	0	13	0	63	3
125.	D(+)-Cellobiose		95	0	100	0	0	0	17	0	25	6
126.	Dextrin		79	23	100	0	0	32	50	28	0	0
127.	Dulcitol		0	0	0	0	0	0	0	0	0	9
128.	Erythritol		3	0	0	0	0	0	0	0	0	0
129.	D(-)-Fructose		100	100	100	100	100	100	100	100	100	100
130.	Galactose		82	54	0	100	100	100	92	100	0	94
131.	Glucose		100	100	100	100	100	100	100	100	100	100
132.	Glycerol		84	85	0	33	30	63	57	86	13	11
133.	Glycogen		5	8	100	0	10	16	0	13	0	0
134.	meso-Inositol		0	0	0	0	70	0	71	28	25	0
135.	Inulin		0	0	0	0	0	0	0	0	0	3
136.	Lactose		100	100	100	0	20	42	33	28	0	9
137.	Maltose		97	100	100	67	60	100	100	100	100	20
138.	Mannitol		5	100	0	100	89	100	100	0	100	86
139.	D(+)-Mannose		100	92	100	100	100	100	17	100	100	100
140.	D(+)-Melezitose		0	0	0	0	10	5	0	0	0	0
141.	D(+)-Melibiose		100	92	100	100	0	0	0	28	0	0
142.	Methyl D-glucoside		3	0	0	0	0	0	0	13	0	0
143.	Methyl D-mannoside		0	0	0	0	0	0	0	0	0	3
144.	N-Acetylglucosamine		100	92	100	100	100	57	96	100	100	100
145.	D(+)-Raffinose		100	100	100	0	0	26	46	57	0	3
146.	Rhamnose		0	0	0	0	20	0	0	0	0	0
147.	Ribose		100	100	100	67	89	100	100	100	88	97
148.	Salicin		100	0	100	67	0	0	17	0	63	9
149.	Sorbitol		0	23	0	33	89	16	96	0	100	77
150.	L(-)-Sorbose		0	0	0	0	0	0	0	0	0	0
151.	Starch		13	0	0	0	0	0	8	0	0	0
152.	Sucrose		100	100	100	33	100	100	96	100	100	97
153.	D(-)-Trehalose		100	100	100	100	20	0	0	100	100	23
154.	D(+)-Xylose		100	100	100	0	100	100	96	71	0	73
155.	L(-)-Xylose		24	0	0	0	10	26	33	43	0	17

Phenons 18 and 19 are satellites that are indole positive, inositol positive, and give gas in Hugh & Leifson medium.

Phenon 20 corresponds to *Pasteurella aerogenes* as it contains the type strain A221, but also strains with other names (see Discussion). Strains grow on violet red bile agar, and with malachite green, crystal violet and 3% NaCl. They acidify sucrose, galactose and D(+)-xylose. Phenon 21 is a satellite that is unusual in acidifying sorbose and L(-)-xylose.

Phenon 22, referred to as the 'aer-2' groups, is a small group of aerogenic strains quite similar to phenon 20. It is characteristically positive for dulcitol, starch and rhamnose. Phenon 23 is a satellite notable for acidifying starch, and it is catalase negative.

Table 3 (continued)

11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
0	0	0	0	0	0	82	0	0	77	0	100	100	100	25	33	100	0	100	33	50	0
0	0	0	0	13	0	0	NT	NT	0	0	0	0	0	0	0	0	0	NT	0	50	0
0	0	0	0	100	100	0	NT	NT	100	100	100	100	100	50	100	0	0	NT	0	25	0
NT	0	0	0	0	0	0	0	0	31	0	80	0	0	0	71	0	0	0	0	0	NT
NT	0	75	0	0	0	27	100	100	31	0	100	100	0	0	0	0	0	0	0	0	0
100	NT	100	80	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
0	NT	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100
0	NT	75	0	0	0	100	100	100	31	100	100	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0
0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
0	0	0	0	0	0	27	100	0	23	100	100	0	100	0	43	0	0	100	0	0	0
0	0	0	0	0	0	100	100	100	62	100	100	100	100	100	100	100	100	100	33	0	0
0	0	0	0	0	0	9	0	0	8	0	0	0	0	50	67	100	100	100	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0	0	0
57	0	0	0	13	0	0	100	0	8	100	100	100	0	0	5	9	0	100	0	25	0
0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	100	0	0	0
100	100	75	80	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	75	0
100	0	0	0	0	100	91	100	0	100	100	100	100	100	100	100	100	100	100	0	75	0
100	100	75	20	100	100	91	100	100	100	100	100	100	100	100	100	100	100	100	0	75	33
0	0	0	0	0	0	82	100	0	46	100	100	100	50	100	100	100	100	0	0	50	0
33	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	9	0	0	0	50	0
0	0	0	40	0	0	100	100	100	39	0	0	100	100	50	86	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	36	100	0	15	100	100	100	50	0	10	0	0	0	0	50	0
100	100	25	0	86	0	18	100	100	92	100	100	100	100	0	67	100	100	100	0	50	0
100	0	0	0	100	0	100	100	100	23	0	0	0	100	100	100	100	100	100	0	0	0
100	100	75	0	28	100	27	0	0	92	100	100	100	0	100	100	100	100	100	0	75	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0
0	0	0	0	0	0	0	0	0	39	100	100	100	0	0	0	82	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	25	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
100	0	75	0	71	100	0	0	0	92	0	100	100	100	100	95	100	100	100	0	0	0
0	0	0	0	0	0	18	0	0	46	100	100	100	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	73	0	0	8	100	100	0	0	0	0	100	100	0	0	0	0
100	0	50	20	86	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	75	33
0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	38	91	100	100	0	0	0
100	0	0	0	13	0	100	100	100	15	0	0	0	0	50	100	0	0	50	0	0	0
0	0	0	0	0	0	0	0	0	0	100	0	0	0	50	86	0	0	0	0	0	0
0	0	0	0	0	0	0	100	0	0	0	100	100	0	0	0	0	0	0	0	0	33
0	0	0	0	100	100	0	100	100	100	100	100	100	100	50	100	9	0	0	0	75	0
0	0	0	0	0	0	0	0	0	23	100	0	0	0	50	95	100	100	100	0	50	0
0	0	0	0	0	0	91	100	100	100	100	100	100	100	50	57	91	0	100	0	0	0
0	0	0	0	0	0	9	0	0	8	100	20	0	0	0	0	0	0	10	0	0	0

Phenon 24 contains two replicates of a strain from a tortoise, and is referred to as the 'tortoise' group (see Discussion). These strains grew in 5% NaCl and were resistant to azide and arsenate; they grew at 15 °C and acidified D(–)-arabinose.

Area B: yersinias (phenons 25–29).

Phenons 25 and 26 comprise strains of *Yersinia enterocolitica*. Although our study did not include the type strain (ATCC 9610), there is little doubt that phenon 26 corresponds to classical *Y. enterocolitica*, and phenon 25 (referred to as the 'ent-b' group) consists of strains that are less typical (see Discussion). These bacteria are characteristically able to grow at 10 °C and on

deoxycholate-citrate agar, and with acriflavine and copper sulphate. They acidify cellobiose, but seldom hydrolyse aesculin.

Phenon 27 is *Yersinia pseudotuberculosis* and phenon 28 appears to be a typical strain of this species. These phenons grow at 10 °C and on deoxycholate-citrate agar, and with acriflavine and copper sulphate, but also with tellurite. They are aesculin positive.

Phenon 29 (*Yersinia pestis*) is represented by replicates of only one strain, but it is notable for acidifying erythritol and L(–)-xylose. These two test results are, however, not typical of the species (Bercovier & Mollaret, 1984).

Area C: miscellaneous (phenons 30–32).

These three groups are non-fermentative and produce alkali in Hugh & Leifson medium. They are the most isolated in the phenogram (Fig. 1).

Phenon 30. This miscellaneous group, referred to here as 'past-b', contains three strains which are not very closely allied to each other or to other phenons (see Discussion). They grow both in 7% NaCl and with copper sulphate.

Phenon 31 (*Yersinia philomiragia*) is characteristically positive for gelatinase and is lipolytic on Tweens. Strains grow on 8% NaCl and produce H₂S in triple sugar iron medium.

Phenon 32 is *Moraxella anatipestifer*. Strains are positive for DNAase, sensitive to janus green and to Teepol, but show some lipolysis of Tweens.

Distinctness of phenons and their relationship in phenetic space

Compactness and overlap of phenons. The phenons vary considerably in compactness (Fig. 1 and d_j in Table 2), from *P. aerogenes*, phenon 20, the least compact, to the most compact, phenon 29 (*Y. pestis*). The values for some phenons are based on few strains, or are based on replicates, and so they may reflect mainly lack of test reproducibility (e.g. phenons 24 and 29), but it may be noted that *P. multocida*, which has traditionally been considered a rather variable species, is indeed among the less compact. The high variability of phenon 8 is partly accounted for by containing *P. pneumotropica* and *P. gallinarum* which are probably distinct (see Discussion).

The overlap statistics show that the great majority of the phenons are quite distinct. Only six pairs showed a nominal overlap, V_G , of more than 1%; the phenon pairs 7:32, 8:10, 8:20, 10:11, 10:32 and 25:26. All these showed overlap of less than 5%, except for the last, and the small number of strains prevents a satisfactory test of significance. The last, however, showed about 17.6% overlap, which is considered in the Discussion.

Principal coordinates analysis. A general view of the major relationships of the phenons is shown in Fig. 2. It should be noted that ordinations such as Fig. 2 do not reflect exactly the same aspects of multidimensional relationships as phenograms, but in this instance, the discrepancies are small. The main groupings of the phenogram are reflected in Fig. 2. Phenons of group A (phenons 1–24) lie mainly at the back and to the left: these represent the actinobacilli and pasteurellae. Phenons of group B (25–29, the yersinias) lie to the front and left. The group C phenons (30–32) lie to the right: these represent the most atypical organisms, and they are joined in Fig. 2 by phenons 12 (a satellite) and 14 ('*A. seminis*'), which are also very different from most of the other groups.

The variance on the first three axes (indicated by the eigenvalues) is just over 40%, somewhat low for such analyses (Bridge & Sneath, 1983). The sum of the first eight eigenvalues represents 63.12% of the total variation. Phenons with high metabolic activity lie to the right in Fig. 2, and those with low activity to the left, as shown by the vigour values (Table 2).

Other findings

Single Linkage clustering with S_G . Single Linkage (cophenetic $r = 0.74$) showed similar structure to the UPGMA clustering in Fig. 1. The strain membership of phenons was broadly the same, and identical in many, but a few strains of some phenons moved out into poorly clustered regions of the phenogram. One such contained a few strains from phenons 2 to 7. A subcluster of *A. equuli* (A255–258) moved close to *P. haemolytica* biovar A, and *A. lignieresii* also appeared in two subgroups, not well separated from *A. equuli* and *P. haemolytica* biovar A. The

Fig. 2. Principal coordinates analysis of the distances between centroids of the 32 phenons. The phenon numbering is the same as in Fig. 1. The intercentroid distances and radii of the phenons are from Table 2. The former were scaled by multiplying by $\sqrt{155}$ and the latter were obtained by multiplying d_i by $\sqrt{(3/155)}$, so as to take account of the reduction from 155 to 3 dimensions. The first principal axes are shown, with the baseplate lying at -3 on the vertical axis, III. The corresponding eigenvalues are 96.48, 66.42 and 42.05, corresponding to 40.46% of the total variation.

strains of *P. aerogenes* (phenon 20) and of phenon 8 were often mingled and showed poor clustering. The arrangement into the groups A, B and C was, however, broadly preserved.

Vigour and Pattern Coefficients. The vigour of the phenons (which largely reflects metabolic activity) varies a good deal (Table 2) from the metabolically inactive '*A. seminis*' and *Moraxella anatipestifer* to the metabolically active yersinias. Among the latter, as expected, phenon 25 contains strains similar to *Y. enterocolitica* but of lower vigour (see Discussion).

There is no marked difference in vigour between the accepted species of *Actinobacillus* and *Pasteurella*, although if *A. actinomycetemcomitans* is excluded the actinobacilli show higher values than most of the pasteurellae. *P. aerogenes* (and also the similar strains in phenon 22) shows high vigour values, however. *P. haemolytica* biovar T is notably less metabolically active than biovar A. In this respect, the 'BLG' and '*P. bettii*' groups are more like classical pasteurellae than classical actinobacilli.

The UPGMA phenogram with the Pattern Coefficient (cophenetic $r = 0.81$) showed phenons with almost the same members as that with S_G . The main differences were as follows. The satellite phenon 3 moved near the yersinias. Phenon 8 moved near *P. multocida*, as did *A. actinomycetemcomitans*. *P. ureae* was closer to *A. equuli*. The aerogenic phenons were somewhat rearranged: *P. aerogenes* divided into two, one (including the type strain) was combined with '*P. bettii*'. The areas A, B and C were preserved except that phenon 4 ('BLG') became attached to the yersinias.

It is usual to find with the Pattern Difference (D_p) that some satellites move into well-defined clusters because they are slow-growing variants whose slower growth leads to differences from typical members of their species, and this is corrected by using the D_p coefficient. In this study, however, only the satellite A241 (phenon 16) behaved in this manner; it moved into *P. aerogenes*, and is presumably a member of that or of a closely related species.

The phenon 'aer-2' (phenon 22) remained a separate group with D_p , with A163 (phenon 12) as a satellite. A few strains moved in an unexpected way: '*A. seminis*' A280 moved into *A. suis*, and *P. ureae* A87 moved into *A. equuli*, while *A. equuli* A264 moved into *P. ureae*. These indicate that the pairs of taxa involved are rather similar in phenotype if the vigour component is removed, i.e. '*A. seminis*' has positive properties similar to *A. suis*, and *P. ureae* to *A. equuli*.

The Single Linkage phenogram with D_p (cophenetic $r = 0.68$) was similar to the Single Linkage phenogram with S_G and no special points of interest emerge: again the region of *P. aerogenes*, *P. pneumotropica* and *P. gallinarum* was not well-clustered.

Cophenetic discrepancies. Most of the distortion between a similarity matrix and a phenogram derived from it is due to a few of the OTUs (Sneath & Sokal, 1973; p. 282). As has been found in earlier studies (Sneath *et al.* 1981; Bridge & Sneath, 1983), these 'poorly fitting' OTUs are commonly satellite strains or those on the edge of their cluster or from the most peripheral clusters. The highest distortion Δ_j in the S_G UPGMA analysis was shown by strains of *M. anatipestifer*. Strains with high Δ_j tended to have vigour values that were either very high or very low. This agrees with the findings of Sneath *et al.* (1981), though Bridge & Sneath (1983) found little association between high Δ_j and low vigour.

Identification statistics. The percentages in Table 3 were treated as an identification matrix and evaluated by computer programs. The program MOSTTYP showed that all phenons were readily differentiated by the 155 characters in that identification scores by the most typical possible strain (hypothetical median organism) were always excellent when compared with its own phenon, and much better than the score to the phenon of second choice. The sum of character scores from DIACHAR showed satisfactorily high values for all phenons, ranging from 38.5 for the least well defined, phenon 20, to 63.5 for the best defined, phenon 21 (excluding higher values for phenons containing only one strain). The properties that were most useful for differentiating the phenons, from CHARSEP, included acid from mannitol, L(+)-arabinose, D(-)-xylose, trehalose, glycerol, sucrose, galactose and sorbitol, and also urease, growth on 1% Teepol, nitrite destruction, oxidase, ONPG, growth on violet red bile agar and on 3% NaCl and hydrolysis of aesculin. The results from OVCLUS were confirmed by the program

OVERMAT, which showed that all phenons as represented by an identification matrix, were distinct; all possible pairs showed overlap of less than 1% at the 90% probability level wherever the numbers of strains allowed significance to be tested. The fact that the nominal overlap of phenons 25 and 26 fell below 1% presumably reflects the assumption used in the OVERMAT program that the clusters are hyperspherical, which may exaggerate the apparent distinctness between groups.

DISCUSSION

Test reproducibility

Replicates on the same batch of medium showed, as expected, rather better test reproducibility than those on different batches, although the difference is not marked. It was unexpected to find better agreement between the same strains from two sources than between deliberate replicates, but in view of the small numbers of strains, this may not be significant. Differences in vigour on the two batches were small. The behaviour of strains of phenon 27 in the two batches of media remains unexplained: there was no difference in the sets of tests, and we presume small changes in test conditions were responsible.

The properties of the phenons agreed reasonably well with those tabulated by Kilian & Frederiksen (1981), Sakazaki *et al.* (1981), Carter (1981, 1984) and Phillips (1981, 1984), if allowance is made for the fact that the carbohydrate fermentations and a few other tests in the API kits were read early, after 24 h incubation. Also, the conditions in the API kits are not the same in some respects as in conventional tests. Major discrepancies of over 30–40% are briefly mentioned below, with comments on a few tests where testing conditions are particularly important.

The carbohydrates that were less often acidified in the API kits than expected from the literature (with the relevant phenons in parentheses) included raffinose, melibiose, glycogen and starch (phenons 7 and 9), inositol and salicin (phenon 9), and dextrin (phenons 2, 6, 9 and 20). ONPG was less often hydrolysed than expected (phenons 2, 6, 20 and 26). Cases where acid was produced where none was expected were few: occasional strains acidified sorbitol (phenons 2, 6, 11 and 20), salicin, mannose and cellobiose (phenon 7), mannitol (phenons 1 and 20) and trehalose and raffinose (phenon 20). Weak reactions by phenons 31 and 32 are considered later. We found strains of phenon 4 to acidify galactose and mannose, whereas Jayaraman & Sethumadavan (1974) found they did not.

In the API galleries the carbohydrate fermentations at 48 h were usually unchanged from 24 h. The major increases, over 20%, in percentages of positives were as follows: phenon 6, dextrin rose to 63%; phenon 7, raffinose rose to 77%; phenon 9, raffinose rose to 25%; phenon 26, ONPG rose to 65%.

Sticky colonies with pronounced production of mucus were noted for some strains, particularly of phenons 1, 2, 7, 9 and 31, and some of these gave the appearance of being capsulated. These findings, however, were often equivocal, and we did not therefore score them as characters.

The ability to grow on MacConkey agar is affected by the batch or formulation. We used Oxoid MacConkey agar with the usual amount of NaCl (0.5%), which contains 0.5% (w/v) bile salts, but several formulae are commonly used. This may explain why *P. multocida*, and often *P. ureae*, grew. Similar variability was noted by Sakazaki *et al.* (1981). It would seem better in future to test resistance to a purified bile salt such as sodium taurocholate or deoxycholate.

Methyl-red and Voges-Proskauer reactions did not always agree with the literature. In our hands, a number of phenons were often positive for both tests at 37 °C, though rarely at 25 °C or in API kits. These tests, however, are much affected by test conditions, which are often not specified in the literature, and Sakazaki *et al.* (1981) noted similar variability. There were some discrepancies in indole production: we found that *P. multocida* was sometimes negative and *P. aerogenes* sometimes positive, and *Y. philomiragia* was negative (see Bercovier & Mollaret, 1984). It is possible that some strains received as *P. aerogenes* are strains of *P. pneumotropica* (which is usually indole positive); the present confusion over these names suggests this should be

re-investigated. Phenons 6, 10 and 17 were seldom positive as expected for H₂S (lead acetate paper). We found that *Y. philomiragia* was oxidase positive and *P. aerogenes* was variable.

Destruction of nitrite depends critically on the test conditions. Our results with 0.001% nitrite agree with the data of Kilian & Frederiksen (1981) except that most strains of phenon 10 destroyed it, but with the higher level (0.01%) fewer phenons were positive. The API urease test was less sensitive than that of Cowan (1974), and strains of phenons 2, 6, 15 and 20 were less often positive in the former.

Major discrepancies were seen in the production of gas from glucose (both in glucose broth and in Hugh & Leifson medium). However, Kilian & Frederiksen (1981) incubated for up to 30 d. Nevertheless, the clear clustering of *P. aerogenes*, even though most strains did not produce gas within 2 d, shows that these groups do not turn critically on gas production.

The identification matrix of Bascomb *et al.* (1973) was not constructed from a numerical taxonomy, so some differences might be expected. We found glycerol was more often acidified than in their matrix, and a few other major percentage discrepancies were found, probably due mainly to the use of API galleries in our study.

The matrix evaluation programs showed that the present 155 characters would allow construction of a reliable computer-assisted identification system. In practice, many tests would be omitted because of the difficulties of making up special media. It would be most convenient to build the system around manufactured kits such as API galleries, but it may be noted that a rich medium like BMB is needed for these bacteria. Useful new tests are suggested by CHARSEP and DIACHAR. These include those based on the following inhibitory compounds (see Methods for concentrations): Teepol, 4,4'-diamidinodiphenylamine, crystal violet, sodium azide, copper sulphate, janus green, malachite green and potassium tellurite. Growth on 3% NaCl and violet red bile agar, and lipolysis of Tween 60, may also be useful.

Phenons

Area A: phenons 1-24. Strains of *A. suis* (phenon 1) are usually from swine, but also from horses (Biberstein, 1981); three of our strains came from horses. Frederiksen & Kilian (1981) also mention records from humans. Strains A63 and A64, though received as *A. lignieresii*, are typical *A. suis* and appear to be the *A. suis* strains of Wetmore from irradiated swine referred to by Phillips (1981). We observed slime in stained films less often than expected for this species, and did not note yellow pigment, although this requires special conditions for its observation (Phillips, 1984).

Phenon 2 (*A. equuli*) is usually associated with septicaemia and joint infections in foals, but may occur in swine (Phillips, 1984), as some of ours did. Phenon 3 remains unidentified. Phenon 4, the 'BLG group', is distinct, though only a few strains were studied. Our results agreed well with those of Jayaraman & Sethumadavan (1974) except on galactose and mannose. They were uncertain of the relations to *A. lignieresii* and *Y. pseudotuberculosis*; there was some evidence of shared antigens with both of these species. These bacteria cause lymphangitis of cattle (*Bos indicus*) in southern India. It may be noted that phenon 4 in Fig. 2 is close to the yersinias, as it was in the UPGMA phenogram from the Pattern Coefficient. We have not been able to identify phenon 4 with an existing species. Strain A82 was distinctly related to other species in the DNA pairing study of Pohl (1981). Phenon 4 may deserve species status.

Phenon 5 ('Mair' group) is another well-defined group. Its veterinary significance is not clear, though it has been associated with abortion of sows and piglet pneumonia (N. S. Mair, personal communication). Pohl (1981) found that strain A10 was distantly related to A221 (*P. aerogenes*). This phenon may also deserve species status. *A. lignieresii* (phenon 6) requires little comment. Though found mostly in cattle and sheep, it occurs in the horse (Phillips, 1984), and the equine isolate A58 is a typical strain. However, we subsequently have been told by W. Frederiksen (personal communication) that the organisms from porcine pleuropneumonia of Bertschinger & Seifert (1978) appear to be strains of *A. lignieresii*, and that their strains are A283, A284, A285 and A286 in this study. They are reported as being similar to *Haemophilus pleuropneumoniae* but do not require V factor (Pohl *et al.*, 1983), but in our study they lie unambiguously within phenon 6.

Phenons 7 and 9, the A and T biovars of *P. haemolytica*, are clearly very different. The nominal overlap was 6×10^{-4} , so the phenotypic evidence supports the belief they should be separate species with the epithet *haemolytica* for biovar A, which contains the type strain. The numerical taxonomy of Sakazaki *et al.* (1981) and the DNA pairing data of Pohl (1981) are in keeping. Biberstein & Gills (1962) had noted a sharp separation in the serovars of these strains into the A and T forms, and Biberstein & Francis (1968) showed they differed on DNA pairing. We found that A104, though received as biovar T, serovar 10, had all the reactions of biovar A. There was no evidence for a subphenon within phenon 7 for the strains bearing the name '*P. mastitidis*' described by Marsh (1932).

Phenon 8. We had an inadequate number of strains of this phenon. The type strains of both *P. pneumotropica* and *P. gallinarum* occur in it, and the strains fall into two corresponding subphenons at about 84% S_G . More extensive study would probably have shown separate species, in keeping with the findings of Sakazaki *et al.* (1981) and Pohl (1981). The main differences between the subphenons were as follows: the *P. pneumotropica* subphenon was predominantly urease positive, ONPG positive, dextran negative, resistant to azide and sensitive to borate, whereas the *P. gallinarum* subphenon showed predominantly the opposite reactions. The present data do not clarify the relationships between biovars of *P. pneumotropica* discussed by Frederiksen (1981) and Bisgaard *et al.* (1983). We did not have a representative of the 'Heyl' biovar, but A80 and A86, belonging respectively to the 'Henriksen/gas' and 'Jawetz' biovars (the latter being the type strain) are both in phenon 8. There are rather few differences between these biovars, and between them and *P. gallinarum*, but strains of *P. aerogenes* are evidently sometimes misidentified as *P. pneumotropica* (Table 1, phenon 20).

P. multocida (phenon 10) has in the past been considered a very variable species. Bascomb *et al.* (1973) separated some strains of *P. multocida* into a separate 'biovar 2' because of the difficulty of identifying them with classical *P. multocida*. We could not identify biovar 2 with any of our phenons, but strains with similar properties occurred in phenons 8, 16 and 23. In this study *P. multocida* is rather diverse but not particularly so (Table 2). In earlier days phenotypically similar forms had not been recognized as distinct species (e.g. *P. pneumotropica*, *P. aerogenes*). The synonym *P. gallicida* has now been suppressed (Judicial Commission of the International Committee on Systematic Bacteriology, 1985).

Pohl (1981) found high DNA pairing between strains that are probably our strains A85, A109, A113 and A114. We did not have a representative of the distinct 'dog-type biovar 6', unless phenon 16 represents this (see below). We found, like Sakazaki *et al.* (1981), that L-arabinose negative, D-xylose positive strains were of avian origin, but the cluster analysis showed no clear subphenons. Representatives of serogroups A to E of Carter (1963) all came in phenon 10.

Phenon 11 strains (*A. actinomycetemcomitans*) grew rather poorly, which may account for their low vigour (Table 2). This species is common in the human mouth and may be implicated in dental plaque (Frederiksen & Kilian, 1981). It is distinct in the studies of Sakazaki *et al.* (1981) and Pohl (1981). Phenon 12 was received as a strain of '*P. anatipestifer*', but differs in a number of ways, and may have been misidentified. Phenon 13 ('*P. bettii*') is quite distinct. It has been little studied, and might be revived as a species name if more strains could be found. Pohl (1981) placed '*P. bettii*' near *Haemophilus parainfluenzae* on DNA pairing. Phenon 14 ('*A. seminis*') has also been little studied. Pohl (1981) found strain A65 was distant from all of his other species. These strains produced very little acid from carbohydrates and though we thought they were weakly fermentative in Hugh & Leifson medium, this requires confirmation. '*A. seminis*' causes epididymitis in rams. One strain received as '*A. seminis*' (A165) clustered in phenon 30 and is clearly phenotypically different from the strains in phenon 14. It is noteworthy that Van Tonder (1973) observed that A164 (his strain 6201) and A166 (70-84) were serologically very similar and that A165 (T981V) was serologically different. His serological findings therefore confirm the phenotypic differences. This suggests that further studies might reveal more than one species causing epididymitis in rams.

Phenon 15 is *P. ureae*. We agreed with Kilian & Frederiksen (1981) that it is usually dulcitol negative, and catalase was difficult to detect in some strains. Henriksen (1961) notes that greening on blood agar may occur at pH 6.8, which may explain the greening we observed. *P.*

ureae is associated with human respiratory tract infections (Frederiksen & Kilian, 1981), and is a separate group in the numerical taxonomy of Sakazaki *et al.* (1981). DNA pairing places it close to *A. suis* and *A. lignieresii*.

Phenon 16 (A241) may represent the 'dog-type' biovar of *P. multocida* (Kilian & Frederiksen, 1981), though unlike this type A241 was indole negative and urease positive. Phenon 17, the 'Ross' group, is a well-defined phenon, associated with swine (Ross *et al.*, 1972). Pohl (1981), on DNA pairing, placed it in the neighbourhood of *A. suis*, *A. equuli*, *A. lignieresii* and *P. haemolytica* biovar A. Two of our strains were aerogenic on first isolation, and several strains gave gas in Hugh & Leifson medium. Phenons 18 and 19 are unidentified satellite strains.

Phenon 20 contains both replicates of *P. aerogenes*; other named strains may have been misidentified. Apart from variability in indole and oxidase, our results agree well with the original description of McAllister & Carter (1974). It has been noted earlier that the low percentage of gas production is probably due to the early time of reading. In agreement with Kilian & Frederiksen (1981) we found the strains were usually aesculin negative. Phenon 21 may represent an atypical strain of *P. aerogenes*. Phenon 22 ('aer-2'), however, appears to be a distinct gas-producing group, which differs only from descriptions of the 'SP' group (Frederiksen, 1981; Kilian & Frederiksen, 1981) in being trehalose negative. Like the SP strains, some of them come from guinea pigs. An organism described by Stewart & Letscher (1976) from guinea pig abscesses is similar. Bisgaard *et al.* (1983) have described four new taxa from guinea pigs. Of these, taxon 5 and taxon 6 are similar to phenons 20 and 22. Taxon 7 is unlike any other group, whereas taxon 8 is similar to *A. equuli*. Their DNA pairing results were not very conclusive, though Ursing (1981) found that *P. aerogenes* and the SP group were well separated by DNA pairing. Phenon 23 may belong to the *P. aerogenes* complex, because it gave gas in Hugh & Leifson medium. Phenon 24 (the 'tortoise' group) is not the same as *Pasteurella testudinis* (see below).

Area B: phenons 25–29. Phenons 25 and 26 contain strains of *Y. enterocolitica* and similar bacteria. Phenon 26 is typical of the species. Phenon 25 resembles *Y. kristensenii* or biovar 5 (Bercovier & Mollaret, 1984), or the sucrose negative, ornithine decarboxylase negative group A4 forms of Kapperud *et al.* (1981), which are separate from *Y. enterocolitica*. It was pointed out by Kapperud *et al.* (1981) that there is an almost continuous gradation from the most metabolically active *Y. intermedia* through *Y. frederiksenii* and *Y. enterocolitica* to *Y. kristensenii*, the least active. This is reflected in the degree of overlap between phenons 25 and 26 (about 17% as measured by the V_G statistic), which is of the order expected from an arbitrary division of a continuous swathe of variation (Sneath, 1977).

Phenons 27 and 28 correspond to *Y. pseudotuberculosis*. Phenon 28 is a close satellite, and is a replicate of a strain in phenon 27; its isolated position is evidently due to lack of test reproducibility. Phenon 29 is *Y. pestis*. This is a very compact phenon because it consists only of a pair of replicates of an avirulent vaccine strain. It does not therefore represent well the diversity of the species, although it does give some indication of phenetic position.

Area C: phenons 30–32. Phenon 30 is a miscellany of strains. They are not closely similar to one another, and may represent poorly growing pasteurellas (see the low vigour value in Table 2) that are inadequately characterized under the conditions of this survey. Strain A165 has been referred to earlier under phenon 14.

Phenon 31, *Y. philomiragia*, a pathogen of muskrats but also found in fresh water (Jensen *et al.*, 1969), and phenon 32, *Moraxella anatipestifer*, which causes septicaemia in ducks (Hendrickson & Hilbert, 1932) are phenetically very different from the other phenons. Strain A131 of phenon 32 is the most isolated strain in the DNA study of Pohl (1981), and the electropherograms of Harry & Brown (1981) are very different from those of other pasteurellas. The taxonomic position of both these species is uncertain. Ursing *et al.* (1980) found no significant DNA pairing between *Y. philomiragia* and other yersinias or with *P. multocida*. *M. anatipestifer* has been regarded as a pasteurella, but present views are that it belongs to neither *Pasteurella* nor *Moraxella* (Bøvre, 1984; Carter, 1984). We found that both phenons 31 and 32 gave very weak attack on a few sugars, and no fermentation in 2 d in Hugh & Leifson medium.

Other taxa. We were unable to identify among our strains phenons representing *A. capsulatus*, '*A. hominis*' or *P. testudinis*. *A. capsulatus* causes joint infections in rabbits (Arseculeratne, 1962). In the description in Kilian & Frederiksen (1981) *A. capsulatus* is similar to *A. suis*, but it is non-haemolytic and acidifies sorbitol, mannitol and amygdalin. Dr W. Frederiksen kindly sent us strains of *A. capsulatus* and '*A. hominis*' which we later examined. *A. capsulatus* NCTC 11408 was in our hands very like *A. suis*, but was non-haemolytic and did not acidify D(+)-arabinose; we found it difficult to demonstrate unequivocal capsules.

In the description in Friis-Møller (1981) '*A. hominis*' is very similar to *A. equuli*, and differs mainly in being mannose negative and fermenting lactose slowly. Christiansen *et al.* (1981) found it close on DNA pairing to *A. equuli* and *P. ureae*. We later examined '*A. hominis*' NCTC 11529, which gave many reactions like those of *A. equuli*, but differed from it in reactions on mannose, glycerol, D(+)-xylose and melibiose.

P. testudinis was described by Snipes & Biberstein (1982) from American desert tortoises. It does not closely resemble phenon 24, which was from a European tortoise. Dr E. L. Biberstein kindly sent us the type strain of *P. testudinis* (ATCC 33688). Its reactions differed from phenon 24 in D(-)-arabinose, rhamnose, melibiose, raffinose, phosphatase and H₂S, and it did not correspond to any of our phenons.

We could not identify any of our phenons with the EF-4 group (Holmes & Ahmed, 1981). Group EF-4 resembles '*P. bettii*' but is positive for oxidase and gelatinase. '*Pasteurella salpingitidis*' of Kohlert (1968) is little known, and does not seem close to other pasteurellas (Pohl, 1981).

Other findings

The phenons in this study are quite compact considering the amount of test error, because this would itself give $\bar{d}_j$ values (Table 2) approaching some of those shown. The replicates composing phenons 24 and 29 would, of course, occupy single points if there was complete test reproducibility. There is a remarkable absence of overlap between phenons (except for phenons 25 and 26 as noted above), and the reduction in degrees of freedom because the variables are binary (Bridge & Sneath, 1983) is not here of practical importance.

The Pattern Coefficient D_p has not shown any special features in this study. Two strains moved into different clusters because the correction for vigour was very marked; a strain of '*A. seminis*' joined *A. suis*, and a strain of *A. equuli* joined *P. ureae*. Such isolated movements have little taxonomic significance, but they do point to pairs of taxa that may be confused if strains grow slowly or tests are read at early times of incubation.

Taxonomic structure of the genera

The phenotypic relations we found are broadly similar to those of Sakazaki *et al.* (1981). Those authors found *A. actinomycetemcomitans* to be further from *P. ureae* than is shown by the present study, but they also noted the relatively isolated position of *P. aerogenes*. The DNA-DNA pairing data of Pohl (1981) are also in reasonable agreement with Figs 1 and 2. Both show *A. suis* close to *A. equuli* and *P. multocida* close to *P. gallinarum*. Pohl (1981) found *P. ureae* was close to *A. equuli*, and *P. gallinarum* was not close to *P. pneumotropica*, but other relations are concordant with his data. Serological relations have been summarized by Carter (1984) and Phillips (1984). There are antigens shared by *A. lignieresii*, *A. equuli*, *A. suis* and *P. haemolytica* biovar A. The quinone composition of these organisms has been extensively studied by Mannheim and his colleagues, and is summarized by Holländer *et al.* (1981), but their occurrence is not easy to interpret taxonomically.

The first two main areas A (actinobacilli and pasteurellas) and B (yersinias) are separated as expected. Area C consists of less closely related non-fermenting forms of uncertain affiliation. Mraz (1969) and Sneath & Johnson (1973) noted that *Actinobacillus* and *Pasteurella* are hard to differentiate and this has recently been discussed by Mutters *et al.* (1984). There are now many more reasons to combine the two genera, and *Pasteurella* would then be the correct name under the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1975; Rule 24b). Within area A there is no clear separation of the two genera (Figs 1 and 2). There is also doubt as to

whether *Haemophilus* is a separate genus. DNA pairing (Christiansen *et al.*, 1981; Coykendall *et al.*, 1983; Potts & Berry, 1983) shows fairly high relationships between some species of *Haemophilus* and *Actinobacillus*, and Pohl *et al.* (1983) have transferred *Haemophilus pleuropneumoniae* to *Actinobacillus* on DNA evidence (see also Kilian & Biberstein, 1984).

Mannheim (1984) and Mutters & Mannheim (1984) have summarized all the DNA evidence. There is a group of species including *P. multocida*, *P. gallinarum* and some strains of *P. pneumotropica*, and perhaps *Haemophilus avium*. Another group contains *A. lignieresii*, *A. equuli*, *A. suis*, *A. capsulatus*, the 'Ross group' and *P. ureae*, and probably *H. pleuropneumoniae*. Both biovars of *P. haemolytica* are close to this group. A third group contains *A. actinomycetemcomitans*, and a fourth contains *H. influenza* and *H. aegyptius*. There are also a number of more isolated taxa.

The present study gives a reasonably clear picture of much of the genera *Actinobacillus* and *Pasteurella*. There is a good deal of uncertainty, however, about the region containing *P. pneumotropica*, *P. gallinarum* and *P. aerogenes*: a detailed study is needed here. It seems very likely that there are a large number of additional groups to be discovered in these genera (similar to the 'BLG' group, for example); these will probably be restricted to one or two animal hosts. Whether they will deserve species status will depend in large part on the extent to which they can be shown to be distinct and not overlapping. For this reason the use of statistical tests of overlap is highly desirable. If the groups overlap too much, it will be difficult to speciate the genera fully, and a system of biovars may then prove more useful.

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