Taxonomic Studies on *Brochothrix*, *Erysipelothrix*, *Listeria* and Atypical Lactobacilli

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One hundred and eighty-seven strains of listeriae, atypical lactobacilli, *Brochothrix*, *Erysipelothrix* and related Gram-positive bacteria were tested for 140 characters based on morphology, physiology and biochemistry. Computer analyses of the data resulted in the recovery of 20 phenons. Representative strains were examined for cytochrome content, cell wall, fatty acid and isoprenoid quinone composition and the G + C content of the DNA. The results indicate that the species *Listeria monocytogenes*, *L. innocua* and *L. ivanovii* are phenotypically very similar. *L. grayi* and *L. murrayi* are more distinct from these but are so similar to each other that *L. murrayi* should be regarded as a subspecies of *L. grayi*. Some of the atypical lactobacilli studied are members of the genus *Brochothrix*; the others probably represent four distinct species in a new genus.

INTRODUCTION

The genera *Brochothrix*, *Erysipelothrix* and *Listeria* all contain Gram-positive, non-sporing, facultatively anaerobic, regular, rod-shaped bacteria, some of which share other common characteristics and in some properties resemble some, as yet, unclassified bacteria referred to as atypical lactobacilli.

Currently the genus *Brochothrix* is monospecific but there is evidence of heterogeneity amongst strains designated *B. thermosphacta* (see Sneath & Jones, 1986). There is also evidence of heterogeneity amongst strains of *E. rhusiopathiae*, until recently the only recognized species in the genus *Erysipelothrix* (see Jones, 1986). While it is now accepted that *L. denitrificans* is not a member of the genus *Listeria*, there is disagreement on the status of the species *L. grayi* and *L. murrayi* (see Seeliger & Jones, 1986). Furthermore, there are few phenotypic characters to distinguish, with certainty, between the other species of the genus *Listeria* (Rocourt *et al.*, 1983*b*; Rocourt & Catimel, 1985).

All three genera, *Brochothrix*, *Erysipelothrix* and *Listeria*, contain bacteria of economic importance. *E. rhusiopathiae* and some listeriae are pathogenic for animals and man; *B. thermosphacta* is a meat spoilage organism. It is therefore important that newly isolated Gram-positive, non-sporeforming, facultatively anaerobic rod-shaped bacteria be identified with confidence. While many such bacteria can be identified as members of the above genera or of the genus *Lactobacillus*, many cannot and these have been referred to as atypical or unclassifiable lactobacilli (Thornley & Sharpe, 1959; Carr & Davies, 1970; Shaw & Harding, 1984).

In an attempt to clarify the taxonomy of the genera *Brochothrix*, *Erysipelothrix* and *Listeria*, and to classify some atypical lactobacilli, numerical taxonomic and some chemical studies were done on these and some representatives of other phenotypically similar bacteria.

METHODS

Strains and growth conditions. Strains and their sources, where known, are listed in Table 1. Type strains were included wherever possible. To monitor the reproducibility of the test system, 14 cultures were duplicated and

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Table 1. Designation, source and cluster assignment of strains plus mol% G + C values of the DNA of some strains

Phenon	Study no.*	Name as received	Source/comment*	Serovar	Mol% G + C
1	NCTC 7973A†	<i>Listeria monocytogenes</i>	Guinea-pig mesenteric node	1a	—
	NCTC 7973B		Guinea-pig mesenteric node	1a	—
	NCTC 5105A		Human	3a	—
	NCTC 5105B		Human	3a	—
	NCTC 10357†		Rabbit	1a	37.0
2	NCTC 7973	<i>Listeria monocytogenes</i>	Guinea-pig mesenteric node	1a	36.9
	NCTC 5348A		Human meningitis	2	—
	NCTC 5348B		Human meningitis	2	—
	NCTC 4885A		Infantile meningitis	4b	—
	NCTC 4885B		Infantile meningitis	4b	—
	NCTC 4885C	<i>L. monocytogenes</i>	Infantile meningitis	4b	—
	NCTC 4885D		Infantile meningitis	4b	—
	NCTC 5214A		Sheep brain	4a	—
	NCTC 4883A		Fowl myocardial disease	4c	—
	NCTC 4883B		Fowl myocardial disease	4c	—
	C342A	<i>Listeria sp.</i>	LRI	—	—
	C342B		LRI	—	—
	NCTC 5214B	<i>L. monocytogenes</i>	Sheep brain	4a	—
	C210	Gram-positive rod	—	—	—
3	C196	<i>L. monocytogenes</i>	PHL	—	—
	C198		Mair, human	—	—
	C199		Mair, human	—	—
	C261		Kahn, 611, non-haemolytic	—	—
	C392		E1, 55/74, non-haemolytic	4g	—
	C398		E1, 645/73, non-haemolytic	4f	—
	C388A		E1, 39-M/74 II, non-haemolytic	4f	—
	C388B		E1, 39-M/74 II, non-haemolytic	4f	—
	C395		E1, 17-J/74, non-haemolytic	4f	—
	C396		E1, 19-J/74, non-haemolytic	4f	—
	C393B	<i>Listeria sp.</i>	E1, 7-J/74, non-haemolytic	4f	—
	C393A		E1, 7-J/74, non-haemolytic	4f	—
	C377		R, 201/SZ, human faeces	III/VI/VII/ /X/XI ABC	—
	C391A		E1, 55/74, non-haemolytic	4g	—
	C391B		E1, 55/74, non-haemolytic	4g	—
	C394		E1, 8-J/74, non-haemolytic	4f	—
	C385		E1, 69-M/73 II, non-haemolytic	1/2	—
	C386		E1, 15-M/74 II, non-haemolytic	4f	—
	C387		E1, 21-M/74 II, non-haemolytic	4f	—
	C378		R, 93/SZ, human faeces	III/V VI XV ABC	—
	C397		R, 96/SZ, human faeces	III/V VI XV ABC	—
	C346†	<i>Listeria sp.</i>	S, NR4203, non-haemolytic	4g	36.4
	C383		Or, 372/73, non-haemolytic	4g	—
	C381		Or, 390/73, non-haemolytic	4f	—
	C382A		Or, 383/73, non-haemolytic	4g	—
	C382B		Or, 383/73, non-haemolytic	4g	—
	C384		Or, 372/a/73, non-haemolytic	4g	—
	C389		E1, 54-M/74 II, non-haemolytic	4f	—
	C390		E1, 80-M/73 II, non-haemolytic	4g	—
	C365		R, 39, human faeces	4ab	—
	C370		R, 23, human faeces	4ab	—
	C368	<i>Listeria sp.</i>	R, 21, human faeces	4ab	—
	C371		R, 27, human faeces	4ab	—
	C369		R, 23, human faeces	4ab	—
	C372		R, 28, human faeces	4ab	—
	C373		Bi, 2862, milk	4a	—

	G142	Gram-positive rod	—	—	—
	C376		R, 17/SZ, human faeces	III/VI/VII/ /X/XI	—
			ABC		—
	C380†	<i>Listeria</i> sp.	R, 75/SZ, human faeces	III/VI ABC	—
	C362†		Fo, 46, human faeces	4ab	—
	C375		R, 21/SZ, human faeces	III/VI/VII/ /X/XI	—
			ABC		—
	C259	<i>L. monocytogenes</i>	Kahn, 560 7169	—	—
	C366		Fo, 84, human faeces	4ab	—
	C344S†		S, NR4205, non-haemolytic	4g	—
	C347G†		S, NR4212, non-haemolytic	4g	—
	C347W†		S, NR4212, non-haemolytic	4g	—
	C348		S, NR4057, non-haemolytic	4f	—
	C349S	<i>Listeria</i> sp.	S, NR4067, non-haemolytic	4g	—
	C350		S, NR4202, non-haemolytic	4g	—
	C530		Wat, L11, sewage	—	—
	C533		Wat, L10, sewage	—	—
	C532		Wat, L4, sewage	—	—
	C531		Wat, L3, sewage	—	—
	C644T†	<i>Listeria innocua</i>	S, no. 3379	6a	36.2
	C534	<i>Listeria</i> sp.	Wat, L5, sewage	—	—
	C535		Wat, L6, sewage	—	—
	C645†	<i>L. innocua</i>	S, no. 3479	6b	36.3
	C367	<i>Listeria</i> sp.	R, 10, human faeces	4ab	36.8
Ungrouped strains	C343†		S, NR4204, non-haemolytic	4g	—
	C363		Fo, 80, human species	4ab	—
	C374	<i>Listeria</i> sp.	R, 19K, human faeces	III/VI/VII/ /X/XI	—
			ABC		—
	C351		S, NR4211, non-haemolytic	4g	—
	C361†		R, 39, human faeces	4ab	—
	C364		R, 81, human faeces	4ab	—
	C197	<i>L. monocytogenes</i>	Mair, 28251, human	—	36.8
4	C659†		P, L242, sheep	5	36.8
	C667†		P, L102B	5	—
	C661		P, L102A	5	—
	C662	<i>L. monocytogenes</i>	P, L133, sheep	5	—
	C663		P, L72	5	—
	C668†		P, L236, sheep	5	35.6
	C664		P, L173	5	—
	C666		P, L234, sheep	5	—
5	C213A	<i>L. monocytogenes</i>	S, L88/7, rough strain	—	—
	C213B		S, L88/7, rough strain	—	—
	C344R†		S, NR4205, rough strain	4g	—
	C345	<i>Listeria</i> sp.	S, NR4206, non-haemolytic	4f	—
	C349R		S, NR4067, non-haemolytic	4g	—
Ungrouped strains	C260	<i>L. monocytogenes</i>	Kahn 611	—	—
	NCTC 5343		—	—	—
6	C214A†		S, 332/64, chinchilla	—	42.4
	C214B†	<i>Listeria grayi</i>	S, 332/64, chinchilla	—	—
	C360†		Seeliger	—	40.5
	ATCC 25401†	<i>Listeria</i>	Corn plant	—	42.5
	ATCC 25402†	<i>murrayi</i>	Corn plant	—	41.2
7	C211†		LTRS MT36, chicken meat	—	35.7
	C494		Sharpe, MT34, chicken meat	—	—
	C496		Sharpe, MT37(L), chicken meat	—	—
	C497†		Sharpe, MT37(S), chicken meat	—	35.4
	C498		Sharpe, MT38, chicken meat	—	—
	C499		Sharpe, MT39(S), chicken meat	—	—
	C503	Atypical	Sharpe, MT42(L), chicken meat	—	—
	C500	Lactobacillus	Sharpe, MT39(L), chicken meat	—	—
	C501		Sharpe, MT40, chicken meat	—	—
	C502†		Sharpe, MT41, chicken meat	—	33.7
	C504		Sharpe, MT42(S), chicken meat	—	—
	C505		Sharpe, MT43(L), chicken meat	—	—
	C506		Sharpe, MT43(S), chicken meat	—	—

Table 1 (continued)

Phenon	Study no.*	Name as received	Source/comment*	Serovar	Mol% G + C
Ungrouped strains	C461	Gram-positive rod	PHL, mouse	—	—
	C471	Atypical <i>Lactobacillus</i>	Sharpe, MT12, chicken meat	—	—
8	C454	<i>Listeria</i> sp.	LRI B480(S), non-haemolytic	—	—
	C523	Gram-positive rod	Chesterfield Hospital, human	—	—
	NCDO 82	<i>Lactobacillus plantarum</i>	—	—	—
	NCDO 1752 ^{T†}		—	—	—
	NCDO 963		—	—	—
9	C462	Atypical <i>Lactobacillus</i>	Sharpe MT2, chicken meat	—	—
	C487		Sharpe, MT27, chicken meat	—	—
	C489		Sharpe, MT29, chicken meat	—	—
	C490 [†]		Sharpe, MT30, chicken meat	—	31.7
	C481		Sharpe, MT21, chicken meat	—	—
	C495		Sharpe, MT35, chicken meat	—	—
	C491		Sharpe, MT31, chicken meat	—	—
	C492		Sharpe, MT32, chicken meat	—	—
	C463		Sharpe, MT3, chicken meat	—	—
	C465		Sharpe, MT5, chicken meat	—	—
	C517 [†]		Sharpe, MT54, chicken meat	—	32.6
	C521		Sharpe, MT58, chicken meat	—	—
	C520		Sharpe, MT57, chicken meat	—	—
	C522		Sharpe, MT59, chicken meat	—	—
10	C507 [†]	Atypical <i>Lactobacillus</i>	Sharpe, MT44, chicken meat ice	—	—
	C508		Sharpe, MT45, chicken meat ice	—	—
11	C464	Atypical <i>Lactobacillus</i>	Sharpe MT4, chicken meat	—	—
	C509		Sharpe, MT46, chicken meat	—	—
	C510		Sharpe, MT47, chicken meat	—	—
	C515		Sharpe, MT52, chicken meat	—	—
	C516 [†]		Sharpe, MT53, chicken meat	—	32.6
	C518		Sharpe, MT55, chicken meat	—	—
	C519		Sharpe, MT56, chicken meat	—	—
	C511		Sharpe, MT48, chicken meat	—	—
	C514		Sharpe, MT51, chicken meat	—	—
	C512		Sharpe, MT49, chicken meat	—	—
	C482 [†]		Sharpe, MT22, chicken meat	—	33.2
	C488		Sharpe, MT28, chicken meat	—	—
	C513		Sharpe, MT50, chicken meat	—	—
	C483		Sharpe, MT23, chicken meat	—	—
	C486		Sharpe, MT26, chicken meat	—	—
Ungrouped strains	C466	Atypical <i>Lactobacillus</i>	Sharpe, MT6, chicken meat	—	—
	C472		Sharpe, MT13, chicken meat	—	—
	NCPPB 83	<i>Corynebacterium insidiosum</i>	<i>Medicago sativa</i>	—	—
12	C1 [†]	<i>Brochothrix thermosphacta</i>	Kitchell, WR7	—	—
	C467	Atypical <i>Lactobacillus</i>	Sharpe, MT8, chicken meat	—	—
	C474		Sharpe, MT15, chicken meat	—	—
	C478		Sharpe, MT18, chicken meat	—	—
	C475 [†]		Sharpe, MT16(S), chicken meat	—	36.0
	C476		Sharpe, MT16(L), chicken meat	—	—
	C477		Sharpe, MT17, chicken meat	—	—
	C479		Sharpe, MT19, chicken meat	—	—
	C480		Sharpe, MT20, chicken meat	—	—
	C493		Sharpe, MT33, chicken meat	—	—
	C2	<i>B. thermosphacta</i>	Kitchell, CR12A,	—	—
	C3 [†]		Kitchell, 30, lamb	—	—
	C209 [†]	Atypical <i>Lactobacillus</i>	LTRS, MT7, chicken meat	—	36.2
	ATCC 11509 ^{T†}	<i>B. thermosphacta</i>	Pork sausage	—	35.6
	C4 [†]		Kitchell, 30A, lamb	—	—

Ungrouped strain	C379	<i>Listeria</i> sp.	R, 96/52, human faeces	III/V VI/XV ABC	–
13	C212 ^{T†‡}	<i>Listeria</i>	CIP, 55134, cooked blood	–	61.7
	ATCC 14870 ^{T†‡}	<i>denitrificans</i>	Cooked blood	–	60.8
14	ATCC 491 ^T	<i>Cellulomonas uda</i>		–	–
	NCIB 8073 ^T	<i>Cellulomonas flavigena</i>	Soil	–	–
Ungrouped strain	C399	<i>Listeria</i> sp.	PHL 12582/75, human	–	–
15	C5	} <i>Kurtha zopfii</i>	Keddie, K2, milk	–	–
	C6		Keddie, K8, meat	–	–
	NCTC 404			–	–
16	C335 [†]	} <i>Erysipelothrix rhusiopathiae</i>	Sneath, 185, mouse	–	60.2
	C338 [†]		Sneath, 182, duck	–	58.2
Ungrouped strains	C469	Atypical <i>Lactobacillus</i>	Sharpe, MT10, chicken meat	–	37.3
	C660	<i>L. monocytogenes</i>	P, L136, sheep	5	–
17	NCPPB 363	' <i>Corynebacterium betae</i> '	Red beet	–	–
	NCPPB 844	' <i>Corynebacterium poinsettiae</i> '	<i>Euphorbia pulcherrima</i>	–	–
Ungrouped strain	NCPPB 797	' <i>Corynebacterium rathayi</i> '	<i>Dactylis glomerata</i>	–	–
18	NCTC 8163 ^{T†}	<i>E. rhusiopathiae</i>	Pig	–	36.0
	C334 [†]	} <i>Erysipelothrix</i> sp.	Sneath, 176, human	–	35.0
	C337 [†]		Sneath, 193, human	–	32.0
19	C484 [†]	} Atypical <i>Lactobacillus</i>	Sharpe, MT24, chicken meat	–	38.6
	C485 [†]		Sharpe, MT25, chicken meat	–	38.8
Ungrouped strain	C524	<i>Erysipelothrix</i> sp.	PHL, pig	–	–
20	C456	<i>Lactobacillus lactis</i>	Sharpe, (L1)	–	–
	NCIB 10560 ^{T†}	} <i>Lactobacillus mali</i>	Cider	–	–
	G144		Carr, 26, cider	–	–
	C672 [†]	<i>Lactobacillus yamanashiensis</i>	Carr, 239, wine	–	–
	C459	<i>Lactobacillus fermenti</i>	Sharpe, (F1)	–	–

T, type strain.

* NCTC, National Collection of Type Cultures; ATCC, American Type Culture Collection; NCDO, National Collection of Dairy Organisms; NCPPB, National Collection of Plant Pathogenic Bacteria; NCIB, National Collection of Industrial Bacteria; CIP, Collection Institut Pasteur; Carr, Dr J. G. Carr, Long Ashton, Bristol; Kahn, Dr Kahn, Sutton Bonnington, Notts; Keddie, Dr R. M. Keddie, University of Reading; Kitchell, Dr A. Kitchell, Cambridge; LRI, Leicester Royal Infirmary; LTRS, Low Temperature Research Station, Cambridge; P, Dr Pritchard, Weybridge; PHL, Public Health Laboratory, Leicester; Bi, E1, Fo, Or, R, S, Dr B. Ralovich, Pécs, Hungary; Seeliger, Professor H. P. R. Seeliger, Würzburg, FRG; Sharpe, Dr M. E. Sharpe, Shinfield, Reading; Sneath, Professor P. H. A. Sneath, University of Leicester; Wat, Mr J. Watkins, Yorkshire Water Authority. Strains suffixed A, B, C and D represent replicate cultures, G and W grey and white colony forms, R and S rough and smooth colony forms. Sharpe MT strains suffixed L and S represent large and small colony forms.

† Strains examined for cell wall amino acids and isoprenoid quinones.

‡ *Listeria denitrificans* has been reclassified as *Jonesia denitrificans* (Rocourt *et al.*, 1987a).

treated as separate strains. Some strains of *Listeria* spp. were obtained from different sources and it was realised later that they were subcultures of the same strain. They were retained in the study because they had different histories and it was of interest to see what effect, if any, this had on their eventual clustering. All the strains were lyophilized and working cultures maintained by weekly subculture on Blood Agar Base no. 2 (Difco). This medium was used as the basal solid medium (BM) and the same ingredients, minus agar, were used when a liquid medium was required. Normally, all tests were inoculated from overnight BM agar cultures. Unless otherwise stated, incubation was at 30 °C, since none of the *B. thermosphacta* strains grew at 37 °C.

Morphology. Cultures (24 and 48 h) on BM agar were examined visually by low-power magnification (×10) under a binocular microscope using reflected and transmitted light and the colony morphology was noted. These

cultures were also inspected for pigment formation. After 2 d incubation, they were left in the light at room temperature for a further 9 d, when pigment formation was determined. Smears of strains grown for 24 and 48 h on BM agar were prepared in saline and Gram-stained by the method of Collins & Lyne (1970) with decolourization by acetone. Cultures (24 h) in BM broth incubated overnight at room temperature (about 22 °C) were examined for motility by the hanging drop method. Pellicle or ring formation, the presence of a deposit and ease of resuspension were examined in tubes of BM broth incubated for 24 and 48 h.

Growth tests. Growth at different temperatures was assessed in tubes of BM broth lightly inoculated with 18 h cultures and incubated in water-baths maintained at 5, 10, 30, 35, 45 and 50 °C $\pm$ 1 °C. Growth was assessed by eye daily for up to 21 d (5 °C), 14 d (10 and 20 °C) and up to 7 d for all other temperatures. Survival at 60 °C for 15 min was tested by lightly inoculating tubes of BM broth (preheated to 60 °C) with 18 h cultures; the tubes were held at 60 °C for 15 min, cooled immediately in ice-cold water, then incubated at 30 °C and examined for growth after 1 and 2 d. For anaerobic growth, duplicate tubes of BM broth were inoculated with 18 h cultures. One was incubated in an anaerobic jar under hydrogen, the other in air. Growth in both tubes was compared and recorded after 5 d.

Growth in BM broth containing NaCl (6.5 and 10%, w/v) was compared with growth in BM broth alone after incubation for 1, 2 and 7 d. Growth on BM agar containing potassium thiocyanate (2.5, 3.75 and 5%, w/v) or thallose acetate (0.01, 0.02 and 0.03%, w/v) or sodium azide (0.01, 0.02 and 0.05%, w/v) was compared with growth on BM agar alone after incubation for 1 and 2 d. Growth and degree of blackening on BM agar containing potassium tellurite (0.1 and 0.5%, w/v; prepared by adding 10%, w/v, potassium tellurite, sterilized by autoclaving, to sterile BM agar) were compared with that on BM agar alone at 1 and 2 d. Growth and reduction of 2,3,5-triphenyltetrazolium chloride (0.01 and 0.1%, w/v; prepared by adding a 10%, w/v, filter-sterilized aqueous solution to sterile SBM agar) (composition given below) were compared with that on SBM agar alone at 1 and 2 d. Sensitivity to nitrite was determined in BM broth containing 0.01% (w/v) KNO₂ dispensed in 7 ml quantities in test-tubes. Growth was recorded after 1 and 2 d. Growth on MRS agar (Oxoid CM 361) and Gardner's (1966) medium was assessed after 1 and 2 d.

Acid from carbohydrates. This was tested in SBM agar which contained: peptone (Oxoid), 1% (w/v); yeast extract (Oxoid), 0.1%; NaCl, 0.5%; agar (Oxoid no. 3), 1.5% (w/v); bromocresol purple, 0.003% (w/v), pH 7.4. Filter-sterilized carbohydrates (with certain exceptions) were added to sterile SBM agar to give a final concentration of 0.5%. The exceptions were dextrin, glycogen and starch, which were autoclaved and then added aseptically to the sterile basal medium. The media were dispensed into repli-dishes (Sterilin), inoculated with overnight cultures as described by Stevens (1969), incubated and examined for acid production after 1, 2, 3, 7, 9 and 10 d.

Carbon source utilization. SBM broth with and without the substrate under test was dispensed in 5 ml amounts into 4 × 0.5 inch (80 × 13 mm) tubes. Both media were inoculated and incubated for 5 d after which growth in the two media was compared by measurement of absorbance at 540 nm. Positive results were scored only when the A₅₄₀ in the medium containing the added carbon source was at least twice that in SBM broth alone.

Antibiotic sensitivities. BM agar plates were lawned with overnight broth cultures. Multodisks (Oxoid) containing appropriate antibiotics were then placed on the plates. Resistance (no inhibition zone was recorded as positive) was assessed after 1 and 2 d.

Degradative tests. Aesculin hydrolysis was determined using SBM broth supplemented with (g l⁻¹) sodium citrate (1), aesculin (1) and ferric citrate scales (0.05). The ferric citrate scales were dissolved by boiling in a small volume of water and added to the medium before sterilization. Blackening of the medium and loss of purple sheen were recorded at 1 and 5 d. Cellulolytic activity was investigated by inoculating strains into tubes of BM broth containing strips of Whatman no. 1 filter paper, half the length of which was above the level of the liquid. The tubes were incubated and examined for filter paper disintegration at frequent intervals for up to 10 weeks. To detect casein hydrolysis an equal volume of 10% (w/v) skim milk (Difco) in distilled water sterilized by autoclaving was added to sterile double-strength BM agar medium. Cultures were streaked to give one line of growth, incubated and the plates examined for areas of clearing for up to 15 d. For detection of lecithinase sterile egg yolk emulsion (Oxoid; final concentration 1%, v/v) was added to cool, sterile BM agar. Cultures were streaked to give one line of growth, incubated and examined for zones of clearing and/or precipitation at 1, 2, 8 and 20 d. Hippurate hydrolysis was detected in BM broth containing 1% (w/v) sodium hippurate. The medium was sterilized by autoclaving, dispensed in 10 ml amounts, inoculated and incubated for 7 d. Each culture was then centrifuged and two 1 ml samples removed. To one was added 1 ml 12% (w/v) FeCl₃·6H₂O; the presence after 30 min of a brown precipitate indicated a positive result. To the other, 1 ml 50% (v/v) H₂SO₄ (Davis, 1955) was added; the presence after 30 min of white crystals was taken as a positive result. Both test methods were used because weak positive reactions are frequently detected only by the H₂SO₄ method. To detect gelatin hydrolysis tubes of BM broth containing 12% (w/v) gelatin (Difco) were stab-inoculated and then inspected at frequent intervals for 14 d. Tubes were refrigerated at 4 °C for 1 h before inspection. Hydrolysis of starch was determined using BM agar containing 2% (w/v) starch. After 15 d incubation plates were flooded with iodine solution and examined for zones of clearing. DNAase and RNAase were detected on BM agar containing 0.08% (w/v) CaCl₂ and 0.2% (w/v) DNA (BDH) or RNA (BDH). After 6 d incubation the plates were flooded with 1 M-HCl and

inspected for zones of clearing. Hydrolysis of Tweens 20, 40, 60 and 80 (Sigma; 1%, v/v, in BM agar) was recorded at frequent intervals for up to 20 d as zones of precipitation and/or clearing. Phosphatase and sulphatase activities were determined using a 1% (w/v) aqueous solution of phenolphthalein diphosphate (sodium salt, BDH) or phenolphthalein disulphate (potassium salt, Koch-Light), sterilized by filtration and added to sterile molten BM agar to give a final concentration of 0.001% (w/v). After 6 d incubation, 1 ml concentrated ammonia solution was placed in the lid of each inverted dish and allowed to stand for 10 min. A positive reaction was indicated by the appearance of a pink colour around the bacterial growth. Tyrosine and xanthine hydrolysis were detected in BM agar containing 0.5% (w/v) L-tyrosine (Sigma) or 0.5% (w/v) xanthine (Sigma), autoclaved and dispensed into Petri dishes. Plates were single-streaked and inspected for zones of clearing at frequent intervals for up to 21 d. Urease activity was detected in a medium consisting of 50 ml 40% (w/v) urea (sterilized by filtration) added to 1 litre sterile molten BM agar, with phenol red (0.012 g) as indicator. This was dispensed in 10 ml quantities into test-tubes and allowed to set as slants. After inoculation the tubes were inspected at frequent intervals for up to 21 d for development of a red colour.

Miscellaneous tests. Haemolysis was detected in layered plates prepared by pouring 10 ml sterile BM agar into Petri dishes; after this had set, a layer (10 ml) of cooled, sterile BM agar containing 7.5% (v/v) horse blood was added. Plates were streaked to obtain single colonies and examined for β -haemolysis after 24 and 48 h. To detect catalase colonies of an overnight BM agar culture were flooded with 20 vol. H_2O_2 and observed for the production of effervescence for up to 5 min. To detect oxidase growth from an overnight BM agar culture was rubbed on Whatman no. 1 filter paper previously soaked in oxidase reagent (Cowan, 1974; solution 1) and observed for the development of a purple colour for up to 10 s (Kovacs, 1956). Oxidation/fermentation of glucose was assessed in tubes of BM broth plus glucose (0.5%, w/v), agar (Oxoid no. 3; 0.4%, w/v) and bromocresol purple (0.003%, w/v). The tubes were inoculated and incubated according to the method of Hugh & Leifson (1953). Gas production from glucose was assessed in SBM broth containing 0.5% (w/v) glucose, dispensed (4 ml) into 4 $\times$ 0.5 inch (80 $\times$ 13 mm) test tubes containing inverted Durham tubes. The inoculated tubes were examined for the production of gas at 1, 2, 5 and 7 d. Gluconate oxidation was assessed in tubes of SBM broth containing 1% (w/v) sodium gluconate. These were inoculated and incubated for 3 d; a Clinitest tablet (Ames) was then added to 0.5 ml of the growth medium. A positive result was indicated by the formation of an orange to brown colour. For the methyl red and Voges-Proskauer tests BM broth containing 0.5% (w/v) glucose and 0.5% (w/v) K_2HPO_4 , pH 7.5, was dispensed in 12.5 ml amounts into 25 ml capacity screw-capped bottles, inoculated and incubated for 7 d. After 3 and 7 d portions were removed and the methyl red test was done as described by Cowan (1974); the Voges-Proskauer test was done after 7 d according to method 1 of Cowan (1974). Ammonia production from peptone was detected using SBM agar containing bromocresol purple (0.003%). This was dispensed into repli-dishes and inoculated as described by Stevens (1969). Dishes were examined for the appearance of a deep purple colour at frequent intervals for up to 10 d. Indole production from tryptone was assessed in medium containing (g l⁻¹) tryptone (Oxoid) (15) plus yeast extract (Oxoid) (1), dispensed into test-tubes in 10 ml quantities. After inoculation the broths were incubated for 16 d and tested by method 2 of Cowan (1974). Hydrogen sulphide production was detected on slants of BM agar supplemented with 0.05% (w/v) cysteine hydrochloride (BDH). These were inoculated and then strips of lead acetate paper were inserted under the cap so that they hung into the tube. The tubes were examined for blackening of the lead acetate paper at frequent intervals for up to 21 d. Arginine deamination was detected by the method of Thornley (1960) with SBM broth as the basal medium. Tests were done with and without a sterile liquid paraffin layer. Results were recorded at 2 and 20 d.

Coding of characters. The data were coded as characters with either two or three states. The two-state characters were coded as positive (1) and negative (0). The three-state characters were coded as strongly positive (2), weakly positive (1) and negative (0). Ten tests which were of no discriminatory value were excluded from the data set. Tests to assess growth on MRS medium and Gardner's medium were not included in the coded data because not all the strains were tested. The results are given separately where appropriate.

Computation. All computing was done on the University of Nottingham ICL 1900 computer, except for the phenograms which were produced on the University of Leicester CDC CYBER 72 using the TAXPAK program written by M. J. Sackin. Gower's similarity coefficient (S_G) (Gower, 1971) was computed and clustering achieved by unweighted pair group average linkage (UPGMA) and single linkage methods (Sneath & Sokal, 1973).

The data were also converted to two-state character form by combining 'strongly positive' and 'weakly positive' into a single 'positive' state scored as 1, with two-state characters unchanged. The simple matching coefficient (S_{SM}) and the pattern difference coefficient (D_P) (Sneath & Sokal, 1973) were then computed on the converted data, and both similarity matrices were clustered by the same two methods. For every clustering, the cophenetic correlation was computed for the dendrogram and similarity matrix from which it was derived.

The IGROUPS (Integer Groups) program printed character value statistics on specified groups of OTUs chosen from the sorted matrix. Overlap statistics were computed by means of the OVCLUS program (Sneath, 1979). The program was run on data for every pair of clusters using the intercentroid distance (D) between each pair of clusters and the standard deviation (s_q) of the q -distributions of each cluster of the pair. The D and s values were obtained from a revised IGROUPS program. The chosen overlap used was 5%.

Cell wall analyses. Most bacteria were grown on BAB2 (Difco) agar plates, incubated at 30 °C for 24 h. *Lactobacillus* strains were grown on MRS (Oxoid) agar plates and *Erysipelothrix* strains in 20 ml amounts of Brain Heart Infusion broth (Oxoid). Fully hydrolysed cell walls were prepared from about 10 mg wet wt of cells by the hot trichloroacetic acid rapid screening method of Schleifer & Kandler (1972). The amino acid composition of complete hydrolysates was determined using an automatic amino acid analyser.

Extraction and analysis of fatty acids and mycolic acids. Selected strains of *Brochothrix* and *Listeria* and atypical *erysipelo*thrix were grown in Brain Heart Infusion broth (Oxoid) with shaking at 30 °C. Cultures were checked for purity at maximum growth, harvested by centrifugation and freeze dried. Fatty acids and mycolic acids were examined using the whole organism methanolysis procedure of Minnikin *et al.* (1975). Fatty acid methyl esters were analysed by GLC (Collins & Jones, 1980).

Extraction and analysis of isoprenoid quinones. Bacteria were grown as described for fatty acid analyses. Isoprenoid quinones were extracted and purified as described by Collins *et al.* (1977) from about 100 mg dried cells and were further examined by reverse-phase partition chromatography (Collins *et al.*, 1980). Mass spectra of the purified quinones were recorded on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 180–200 °C.

Cytochrome composition. *Listeria* strains only were examined for the presence of cytochromes. Cells were grown in Brain Heart Infusion broth (Oxoid) at 30 °C with shaking for 18 h, harvested by centrifugation, washed once in distilled water, and frozen overnight at –70 °C. Cytochrome composition was determined by the procedures of Jones & Redfearn (1966, 1967).

DNA base composition determinations. Representative strains of *Brochothrix* and *Listeria* and some atypical *erysipelo*thrix strains were grown in Brain Heart Infusion broth (Oxoid) supplemented with 0.5% glycine (Fisons) with shaking at 30 °C. Atypical *lactobacilli* were grown in the same medium in stationary culture at 30 °C. Cells were harvested after 12–16 h incubation when in the late exponential or early stationary phase of growth. The method of Garvie (1976) was used for the isolation and purification of the DNA. DNA base composition was estimated by thermal denaturation in standard saline citrate (with the exception of three strains which were examined in dilute saline citrate) by the method of Garvie (1978) but using a Gilford model 240 spectrophotometer.

RESULTS

Numerical taxonomy

Reproducibility. The similarity between duplicate strains varied from 95.7% to 99.6%*S*, with a mean of approximately 98%*S*. This indicates an estimated mean error in testing of 0.975%. The test error was studied by repeating 30 tests (resulting in 33 characters) for all strains and comparing the two sets of data. The reproducibility obtained varied from 100% to 73%. Sneath & Johnson (1972) have suggested that tests showing errors in reproducibility of 10–15% are acceptable. Twelve test characters fell below the 90% reproducibility level and only six below the 85% reproducibility level. Thus, on the whole, both reproducibility between duplicate strains and the reproducibility of tests was good and compared well with figures obtained in other similar studies.

Cluster analysis. (Tables 1 and 2; Fig. 1.) The results of the different computations gave broadly similar groupings. The classification which resulted from the UPGMA clustering of the *S_G* coefficient (Fig. 1) gave the highest cophenetic correlation value (0.94) and the interpretation of the data will be confined mainly to this computation.

All the strains joined at a similarity (%*S_G*) value of 62. The majority of the strains clustered into 20 phenons of very unequal size. The characteristics of the majority of the phenons are listed in Table 2.

With the exception of four strains received as *Listeria* spp. (C454, C379, C399 and C660) and the two cultures of *L. denitrificans*, all strains of *Listeria* clustered in phenons 1 to 6 (overall similarity 80%) or were closely associated with these phenons.

Phenons 1 to 5, *L. monocytogenes*, *L. innocua*, *L. ivanovii* and *Listeria* spp. joined at 88%*S* (Fig. 1). Phenon 1, which contained the type strain (NCTC 10357), can be equated with the species *L. monocytogenes*. Phenon 2 also contained strains that have the properties of this species and like phenon 1 contained haemolytic and non-haemolytic forms. The seven unclustered strains (C343, C363, C374, C351, C361, C364 and C197) were very closely related to phenons 1 and 2, and were non-haemolytic. Phenon 3 contained 58 strains including the type strain of *L. innocua* and all were non-haemolytic. With the exception of the production of acid from xylose by three strains,

Fig. 1. Simplified phenogram showing clusters obtained with S_G coefficient and average linkage algorithm (cophenetic correlation 0.94).

Table 2 (continued)

Phenon no. ...	1-5*	6	7	8	9	10	11	12	13	14	15	16	18
No. of strains ...	99	5	13	4	14	2	15	15	2	2	3	2	3
<i>Growth in presence of (% w/v):</i>													
Nitrite (0.1)	96	100	100	100	100	100	100	100	100	100	100	100	100
Potassium tellurite (0.1)	100	100	92	100	100	100	100	100	50	100	0	0	100
Potassium tellurite (0.5)	100	100	85	50	100	100	100	73	50	0	0	0	100
Potassium thiocyanate (2.5)	100	100	92	100	100	100	100	100	100	100	100	100	100
Potassium thiocyanate (3.75)	100	100	92	25	100	0	80	80	100	50	100	100	100
Potassium thiocyanate (5.0)	100	100	92	0	71	100	53	73	100	50	100	100	100
Sodium azide (0.01)	80	80	100	75	100	100	100	100	100	100	100	100	100
Sodium azide (0.02)	63	80	100	75	100	100	100	73	50	50	100	100	100
Sodium azide (0.05)	26	40	92	75	93	100	60	60	50	0	67	0	100
Sodium chloride (6.5)	100	100	92	75	100	100	100	100	100	100	100	100	0
Sodium chloride (10)	100	100	62	0	100	0	100	100	100	50	67	100	0
Tetrazolium (0.01)	100	100	100	75	100	100	100	100	100	100	67	100	100
Tetrazolium (0.1)	100	100	23	25	100	100	100	100	100	0	67	0	0
Thallos acetate (0.01)	100	100	100	100	100	100	100	100	100	100	100	0	100
Thallos acetate (0.02)	100	100	100	100	100	100	100	100	100	50	67	0	100
Thallos acetate (0.03)	100	100	100	100	100	100	100	100	0	100	100	0	100
<i>Reduction of (% w/v):</i>													
Potassium tellurite (0.1)	99	100	85	0	100	100	100	33	0	0	0	0	100
Potassium tellurite (0.5)	91	100	0	0	100	100	93	33	0	0	0	0	100
Tetrazolium (0.01)	100	100	85	0	100	100	100	100	100	0	67	0	0
Tetrazolium (0.1)	94	60	0	0	100	100	100	73	0	0	67	0	0
<i>Degradation of:</i>													
Aesculin	100	100	0	100	100	50	100	33	0	50	0	0	0
Cellulose	0	0	0	0	0	0	0	0	50	100	0	0	0
DNA	96	100	92	50	100	100	100	93	100	100	100	0	67
RNA	100	100	92	25	93	100	93	93	100	100	100	0	100
Hippurate	100	0	8	75	0	0	40	0	0	0	0	0	0
Starch (aerobic)	11	0	85	25	0	0	0	0	100	100	0	0	0
Starch (anaerobic)	5	0	69	25	0	0	0	0	100	100	0	0	0
Tween 20 (7 d)	69	20	0	0	0	100	27	0	100	100	100	0	0
Tween 20 (14 d)	97	100	0	0	0	100	33	0	100	100	100	100	0
Tween 40 (7 d)	98	20	54	0	50	100	40	0	0	0	100	0	0
Tween 40 (14 d)	100	40	77	0	57	100	60	0	100	100	100	0	0
Tween 60 (7 d)	99	0	38	0	50	100	60	0	50	50	100	100	0
Tween 60 (14 d)	100	40	85	25	57	100	60	13	50	50	100	100	67
Tween 80 (7 d)	39	0	0	0	0	0	0	0	0	50	67	100	0
Tween 80 (14 d)	98	0	0	0	0	0	3	0	0	50	67	100	0
Urea	0	0	0	0	0	0	0	0	0	100	100	0	0
<i>Enzyme activity</i>													
β -Haemolysis	16	0	0	0	0	0	0	0	0	0	0	0	0
Catalase	100	100	0	50	0	0	0	100	100	100	100	100	0
Oxidase	0	0	0	0	0	0	0	0	100	0	0	0	0
Casein hydrolysis	100	100	8	100	100	100	40	100	50	50	0	0	0
Lecithinase	24	0	0	0	0	0	0	0	0	50	0	0	0
Arginine deamination	1	0	100	25	100	100	100	20	0	50	0	0	100
Phosphatase	100	100	0	0	0	0	0	100	0	0	67	0	0
Nitrate reduction	0	40	0	0	0	0	0	0	100	100	0	0	0
Methyl red test	100	100	100	100	100	100	100	100	100	0	0	0	33
Voges-Proskauer test	99	100	0	0	100	0	73	20	0	0	0	0	33
Hydrogen sulphide production	0	40	0	0	0	0	0	0	50	100	100	0	100
<i>Sensitivity to:†</i>													
Gentamycin (10 µg)	100	100	100	100	0	0	80	100	100	100	100	100	0
Kanamycin (30 µg)	100	100	100	75	0	0	33	100	100	100	100	100	33
Nalidixic acid (30 µg)	1	0	0	0	0	0	0	0	0	0	0	0	0
Nitrofurantoin (200 µg)	100	100	100	0	100	100	100	100	100	0	100	50	67
Polymyxin B (300 i.u.)	15	100	100	25	0	0	7	100	100	100	100	100	0
Furazolidone (100 µg)	100	100	54	0	100	100	33	80	0	0	100	33	67
Carbenicillin (10 µg)	100	100	100	75	43	100	33	100	100	100	100	100	100
Colistin sulphate (10 µg)	1	20	15	25	7	0	0	80	0	50	100	0	33

Table 2 (*continued*)

Phenon no. ...	1-5*	6	7	8	9	10	11	12	13	14	15	16	18
No. of strains ...	99	5	13	4	14	2	15	15	2	2	3	2	3
Erythromycin (10 µg)	100	100	100	100	100	100	87	100	100	100	100	100	67
Methicillin (10 µg)	86	100	100	25	7	50	0	0	100	100	100	100	33
Novobiocin (30 µg)	99	100	100	75	71	100	47	100	100	100	100	100	0
Oleandomycin (10 µg)	100	100	100	100	100	100	80	100	100	100	100	100	100
Penicillin G (5 i.u.)	100	100	100	25	71	100	27	100	100	100	100	100	100
Sulphafuraxole (500 µg)	4	0	8	0	0	0	0	7	100	100	33	0	0
Ampicillin (25 µg)	100	100	100	100	64	100	40	100	100	100	100	100	100
Cloxacillin (5 µg)	90	100	31	0	0	0	20	13	50	50	100	100	67
Chlortetracycline (50 µg)	99	100	100	100	93	100	80	53	100	100	100	100	67
Neomycin (10 µg)	100	100	54	75	0	0	7	100	100	100	100	100	0
Oxytetracycline (50 µg)	99	100	100	50	86	100	80	53	100	100	100	100	67
Streptomycin (25 µg)	100	100	92	75	0	0	7	0	100	100	100	100	0
Sulphamethoxazole and trimethoprim (25 µg)	76	60	8	0	29	0	0	27	50	50	0	0	0
Tetracycline (50 µg)	99	100	100	50	86	100	73	53	100	100	100	100	100
Cephaloridine (25 µg)	100	100	100	75	100	100	80	100	100	100	100	100	100
Chloramphenicol (50 µg)	100	100	100	100	100	100	87	100	100	100	100	100	100

* Includes unclustered strains associated with phenons 1-5 (Fig. 1).

† Values in parentheses are the antibiotic concentrations on the Multodiscs.

all are characteristic of the species *L. innocua* (Seeliger, 1981; Rocourt & Catimel, 1985). Where the serovar is known (Table 1), the majority belonged to serovar 6a (formerly 4f, antigen XV), serovar 6b (formerly 4g, antigen XI) (see Seeliger, 1981) or serovar 4ab, serovars found most commonly in strains of *L. innocua* (Seeliger & Jones, 1986).

Phenon 4 contained eight strains that were characteristic of the species *L. ivanovii*. All were markedly haemolytic and none produced acid from mannitol or rhamnose. All were received as serovar 5 (Table 1). All the phenon 5 strains were rough (as determined by colony surface) but could not otherwise be unequivocally distinguished from strains in phenons 1, 2 and 3. Two strains (C260 and NCTC 5343) were attached to phenons 1 to 5. Neither strain was haemolytic, both produced acid from xylose and NCTC 5343 also produced acid from rhamnose. With the exception of the marked haemolysis of strains in phenon 4 and the serotyping of all phenon 4 strains as serovar 5, there was thus no one character that distinguished between these five phenons, the characters of phenons 1 to 5 are therefore given as one set in Table 2.

Phenon 6 contained the type strains of *L. grayi* and *L. murrayi*. Within the phenon there was internal structure that corresponded to two closely related taxa, but one strain received as *L. grayi* (C360) clustered more closely with *L. murrayi*.

Phenon 7 was equivalent to group 3 of Thornley & Sharpe (1959). All the strains were Gram-positive, motile, catalase-negative rods.

Phenon 8 represented *Lact. plantarum*. The apparent closer relationship of these strains to the atypical lactobacilli (phenons 7, 9, 10 and 11) than to other members of the genus *Lactobacillus* (phenon 20) was due mainly to quantitative similarities between the characters of the strains in phenon 8 and phenons 7, 9, 10 and 11. When the simple matching coefficient, which eliminates quantitative differences, was used, the clustering of phenons 7, 9, 10 and 11 remained unchanged but *Lact. plantarum*, while remaining a distinct cluster, showed the closest similarity (phenogram not shown) to the other lactobacilli. This was the only grouping affected by the use of different methods of computation, but it serves to emphasize the importance of using more than one similarity coefficient in numerical taxonomic studies.

Strains previously considered sufficiently similar to be placed in one group (group 2; Thornley & Sharpe, 1959) were recovered in phenons 9, 10 and 11. All were Gram-positive, non-motile, catalase-negative rods. The distinguishing features of the three phenons are listed in Table 2.

Phenon 12 (overall similarity 85%) contained 10 strains designated as group 1 (Thornley & Sharpe, 1959) and five strains of *B. thermosphacta* including the type strain ATCC 11509. Within the cluster there was some evidence of internal structure (not shown in Fig. 1) but inspection of the test results did not reveal any distinguishing characters. The internal structure probably represented strain variation within the species *B. thermosphacta*.

Phenon 13 contained the two cultures of the only known (and type) strain of *Listeria denitrificans* recently reclassified as *Jonesia denitrificans* (Rocourt *et al.*, 1987a). The closest similarity was to phenon 14 (*Cellulomonas*). Strains in both these phenons digested cellulose.

Phenon 15 represented the species *Kurthia zopfii*.

Phenon 16 contained three strains, two of which were received as *E. rhusiopathiae*. However, all were catalase-positive and easily distinguished from the type strain of *E. rhusiopathiae* (Table 2).

Phenon 17 contained one strain of '*Corynebacterium betae*' and one of '*Cor. poinsettiae*'. These have now been reclassified as *Curtobacterium flaccumfaciens* subsp. *betae* and *Curtobacterium flaccumfaciens* subsp. *poinsettiae* respectively (Collins & Jones, 1983). The strains were included in the study because Seeliger (1961) noted that '*Cor. poinsettiae*' could be confused with *L. monocytogenes*. Attached to this phenon at 78%*S* was another plant pathogenic bacterium, '*Corynebacterium rathayi*', now reclassified in the genus *Clavibacter* (Davis *et al.*, 1984).

Phenon 18 contained three strains, including the type strain of *E. rhusiopathiae*. It exhibited a similarity of about 75% to phenon 19 and to one unclustered strain and identified as *Erysipelothrix*. All these strains were catalase-negative.

Phenon 20 contained five strains of various species of the genus *Lactobacillus* (Table 1).

After the completion of the computer analysis all the strains in phenons 7, 8, 9, 10, 11, 12, 19 and 20, and unclustered strains received as *Erysipelothrix* spp., atypical lactobacilli or Gram-positive rods, were tested for growth on MRS medium (De Man *et al.*, 1960), devised for growth of lactobacilli, and STAA agar (Gardner, 1966), a selective medium for *B. thermosphacta*. Good growth on MRS medium was exhibited only by strains of phenons 8 (*Lact. plantarum*) and 20 (*Lactobacillus* spp.), two strains of phenon 19 (C484 and C485) and two unclustered strains (C461 and C471). Only the strains of phenon 12 (*B. thermosphacta*) grew on STAA agar.

Chemical studies

The mol% G + C contents of the DNA of representative strains are listed in Table 1.

Strains of *Brochothrix* and *Listeria* (but not *L. denitrificans*), *Lact. plantarum*, *Lact. mali*, *Lact. yamanashiensis* and those of phenons 7, 9, 10, 11 and 19 all contained *meso*-diaminopimelic acid as the dibasic amino acid. Alanine and glutamic acid were also present. The cell walls of *L. denitrificans* contained lysine, alanine, glutamic acid and glycine. Lysine and alanine were present in all three strains of *E. rhusiopathiae* examined but the presence of glutamic acid, glycine and serine varied between the strains. In contrast, strains C335 and C338 received as '*E. rhusiopathiae*' phenon 16, contained *meso*-diaminopimelic acid and alanine. These two strains also contained mycolic acids.

The major long chain fatty acid of *B. thermosphacta* was 12-methyl tetradecanoic acid (*anteiso*-C_{15:0}) with substantial amounts of 14-methylhexadecanoic (*anteiso*-C_{17:0}) and 13-methyltetradecanoic (*iso*-C_{15:0}) acids. The major fatty acid of *L. denitrificans* was also 12-methyltetradecanoic acid (*anteiso*-C_{15:0}) but with substantial amounts of hexadecanoic (C_{16:0}), 14-methylpentadecanoic (*iso*-C_{16:0}) and 14-methylhexadecanoic (*anteiso*-C_{17:0}) acids. The major fatty acid composition of the genus *Listeria* (phenons 1 to 6, Fig. 1) was 14-methylhexadecanoic (*anteiso*-C_{17:0}) with substantial amounts of other methyl branched acids (Table 3).

No respiratory quinones were detected in representative strains of *Lact. plantarum*, the atypical lactobacilli of phenons 7, 9, 10 and 11, nor in *E. rhusiopathiae*. The remaining strains examined contained menaquinones as the sole respiratory quinones. All *Listeria* (except *L. denitrificans*) and *Brochothrix* strains examined contained MK-7 as the major menaquinone component with MK-6 and MK-5 as minor components. The major menaquinone in *L. denitrificans* was MK-9 with MK-7 and MK-8 as minor components. Strains of *Lact. mali* and *Lact. yamanashiensis* contained MK-8 and MK-9 as major components with MK-10 and MK-7

Table 3. Percentage fatty acid composition of *Brochothrix* and *Listeria* strains

The assignment of fatty acids is as in the following examples. C_{18:0}, octadecanoic acid; C_{18:1}, octadecenoic acid; i-C_{15:0} iso acid 13-methyltetradecanoic acid; ai-15 anteiso acid 12-methyltetradecanoic acid. T, Trace amount. The major fatty acid component is indicated in **bold** type.

Strain	C _{10:0}	C _{12:0}	i-C _{13:0}	ai-C _{13:0}	C _{14:0}	i-C _{14:0}	C _{15:0}	i-C _{15:0}	ai-C _{15:0}	C _{16:0}	i-C _{16:0}	i-C _{17:0}	i-C _{17:0}	ai-C _{17:0}	C _{18:0}	C _{18:1}
<i>B. thermosphacta</i>																
ATCC 11509	T	0.4	T	T	6.4	1.5	T	6.3	69.8	6.2	1.6	-	-	T	6.4	0.1
C1	0.1	0.6	2.5	2.7	2.9	5.1	T	14.8	43.4	3.4	4.2	-	-	1.5	17.8	T
C3	1.0	1.0	0.5	0.4	2.1	2.4	T	16.3	41.0	5.1	4.9	T	T	1.7	22.6	T
C4	0.5	0.5	T	T	2.6	1.8	T	16.7	56.8	4.1	3.5	-	-	0.9	11.8	T
<i>L. monocytogenes</i>																
NCTC 7973	-	0.3	T	T	0.4	1.1	0.7	16.4	27.9	3.9	6.2	T	10.8	31.1	0.1	1.1
NCTC 10357	-	0.3	T	T	0.4	1.2	0.9	16.6	27.7	3.7	6.6	T	10.9	31.0	T	1.3
<i>L. innocua</i>																
C644	-	0.3	T	T	0.3	0.8	0.7	16.5	27.3	3.4	6.3	T	11.1	32.1	T	1.2
C645	-	0.5	0.1	T	1.0	1.9	0.9	16.8	27.0	4.5	6.3	T	11.1	28.7	T	1.2
<i>L. ivanovii</i>																
C667	-	0.3	T	T	0.5	1.1	0.7	16.4	27.9	3.9	6.2	T	11.1	31.1	T	1.1
C668	-	0.3	T	T	0.5	1.2	0.9	16.6	27.9	3.7	6.2	T	10.9	31.0	T	1.3
<i>L. grayi</i>																
C214	0.1	0.4	-	0.1	0.9	3.2	1.6	9.6	23.5	4.3	13.7	T	8.7	32.8	T	1.1
L56/66*	-	0.6	T	T	1.1	3.2	1.8	9.9	22.4	4.2	13.0	T	7.5	34.8	0.6	1.5
W-L96/65*	-	0.2	-	T	0.6	2.5	1.2	10.0	29.2	3.0	13.8	0.3	4.6	33.2	T	1.3
<i>L. murrayi</i>																
ATCC 25401	-	T	0.1	T	0.2	0.6	0.4	12.4	31.9	1.4	6.1	T	3.5	41.9	0.2	1.3
ATCC 25402	0.1	0.6	T	0.1	0.9	1.1	1.3	8.6	28.6	3.7	7.2	T	5.5	40.6	0.2	1.5
ATCC 25403*	-	0.1	T	T	0.4	1.2	1.1	9.9	26.7	3.2	9.5	T	8.4	38.2	0.2	1.1
<i>L. dentrificans</i>																
C212	-	0.5	T	2.6	10.0	4.4	1.8	5.6	31.0	20.0	11.9	T	0.5	11.7	T	-

* Not included in the numerical study

as minor components. The major menaquinone components of the atypical *Erysipelothrix* strains were MK-8(H₂) and MK-9(H₂) with small amounts of MK-7(H₂), MK-8 and MK-9. Cytochrome composition was determined only for *L. monocytogenes* NCTC 7973, *L. innocua* Seeliger L3479, *L. ivanovii* C667 and C668, *L. grayi* Seeliger L332/64 and *L. murrayi* ATCC 25401. All contained cytochromes *a*₁*bdo*.

DISCUSSION

The main aim of this study was to clarify the taxonomy of the listeriae, bacteria designated atypical lactobacilli, and bacteria of the genera *Brochothrix* and *Erysipelothrix*.

Cellulomonas strains were included in the study because bacteria previously known as *L. denitrificans* hydrolyse cellulose and earlier studies had failed to classify them in any known taxon (see Collins *et al.*, 1983). As expected *L. denitrificans* was quite distinct from phenons representing the genus *Listeria*, but while it exhibited the closest relationship to the genus *Cellulomonas*, it cannot be considered a member of that genus. Since this work was completed, *Listeria denitrificans* has been reclassified in a new genus as *Jonesia denitrificans* on the basis of the results of 16S rRNA oligonucleotide cataloguing studies (Rocourt *et al.*, 1987a).

The results obtained with the three plant pathogenic corynebacteria are in agreement with the recent changes in the classification of these bacteria. The strains of '*Cor. poinsettiae*' and '*Cor. betae*', now transferred to the genus *Curtobacterium* (Collins & Jones, 1983) clustered together and showed the closest phenetic similarity to '*Cor. rathayi*', now reclassified in the genus *Clavibacter* (Davis *et al.*, 1984).

The clustering of the strains designated atypical lactobacilli was in broad, but not complete, agreement with the grouping of these strains by Thornley & Sharpe (1959) on the basis of fewer properties. The clustering of all the strains of Thornley and Sharpe group 1 with representatives of *B. thermosphacta* (Fig. 1) together with their cell wall and menaquinone composition and the mol% G + C content of the DNA, indicates that these strains should be designated *B. thermosphacta*. This is in accord with the results of Jones (1975) based on only one strain of Thornley and Sharpe group 1.

In keeping with the results of Thornley and Sharpe (1959), all their group 3 strains formed a distinct cluster (phenon 7). These strains were quite distinct from *B. thermosphacta*. As noted by Thornley & Sharpe (1959) the cell wall peptidoglycan contained *meso*-diaminopimelic acid. However, the strains were catalase-negative and did not contain menaquinones. The apparent similarity of these bacteria to *Lact. plantarum* has been discussed previously; phenon 7 strains were distinct from the lactobacilli and more similar to strains designated group 2 by Thornley & Sharpe (1959).

In contrast to the findings of Thornley & Sharpe (1959), strains allocated by these authors to group 2 formed three clusters (phenons 9, 10 and 11). Comparison of the properties of these strains with those isolated from meat and studied by Shaw & Harding (1985) suggests that strains of phenon 9 resemble strains designated *Lact. carnis* by these authors and strains of phenon 11 bear some resemblance to *Lact. divergens* (Hölzapfel & Gerber, 1983). Neither of these species was included in the study but our results indicate that phenons 9, 10 and 11 are not members of the genus *Lactobacillus*. Moreover, none of the strains grew on MRS medium (De Man *et al.*, 1960). Further work is in progress to resolve the taxonomic position of the strains in phenons 7, 9, 10 and 11.

Of the five strains of *E. rhusiopathiae* studied, two isolates from human sources exhibited properties typical of the species and clustered with the type strain (NCTC 8163) in phenon 18. The other two strains (phenon 16) were received as *E. rhusiopathiae* and one (C335-MEW22) was designated *E. rhusiopathiae* serogroup IV by Gledhill (1945), probably the only strain to be allocated to this serogroup (P. H. A. Sneath, personal communication). The reason for this misidentification is probably because the strains grow very poorly on the usual laboratory media and consequently give a negative catalase reaction. On BM agar, incubated at 30 °C, a positive, but weak catalase reaction was obtained. When 1% (v/v) Tween 80 was incorporated in BM agar very good growth was obtained. Cell wall and menaquinone composition and G + C

content of the DNA indicate that these strains are probably lipophilic corynebacteria similar to *Cor. bovis* (Collins & Cummins, 1986). Very recently a new species of *Erysipelothrix* has been named, *E. tonsillarum* (Takahashi *et al.*, 1987). These bacteria were distinguished from *E. rhusiopathiae* solely on the basis of DNA-DNA hybridization studies.

With the exception of *L. denitrificans* (*J. denitrificans*), the strains received or isolated as members of the genus *Listeria* clustered into six phenons. The high similarity of all these strains (80%) and their identical cell wall, menaquinone, fatty acid and cytochrome compositions indicate that all, including *L. grayi* and *L. murrayi* (phenon 6), are members of the genus *Listeria*.

The clustering of the type strains of *L. grayi* and *L. murrayi* in one cluster indicates that these strains are members of one species. These results do not support the suggestion of Stuart & Welshimer (1973, 1974), based on DNA-DNA hybridization studies, that *L. grayi* and *L. murrayi* should be reclassified in a new genus '*Murraya*'. They do, however, support the suggestion made by these authors that *L. murrayi* should be considered as a subspecies of *L. grayi*. The retention of *L. grayi* subsp. *grayi* and *L. grayi* subsp. *murrayi* in the genus *Listeria* is in agreement with the results of previous numerical taxonomic (Wilkinson & Jones, 1977) and cell wall (Fiedler & Seger, 1973; Fiedler *et al.*, 1984) studies and the recent 16S rRNA cataloguing results of Rocourt *et al.* (1987b).

The recovery of strains received as *L. monocytogenes*, *L. innocua* and *Listeria* spp. (Table 1) in five very closely related phenons and some unclustered strains, is only in partial agreement with the results of the DNA-DNA hybridization studies of Rocourt *et al.* (1982). These authors detected five genomic species among strains previously classified as *L. monocytogenes sensu lato* (Rocourt *et al.*, 1982), *L. monocytogenes sensu stricto*, and species subsequently validly published as *L. innocua* (Seeliger, 1981, 1983), *L. welshimeri* (Rocourt & Grimont, 1983), *L. seeligeri* (Rocourt & Grimont, 1983) and *L. ivanovii* (Seeliger *et al.*, 1984). These genomic species were distinguished by a few phenotypic tests (Rocourt *et al.*, 1983b), and a further more extensive study (Rocourt & Catimel, 1985) failed to detect any further distinguishing properties. Reference strains of *L. welshimeri* and *L. seeligeri* were not included in the present study nor was the CAMP test with *Staphylococcus aureus* and *Rhodococcus equi* done initially. Examination of the strains in phenons 1-5 by the CAMP test (Rocourt *et al.*, 1983b) showed that all strains in phenons 1 and 2 were CAMP positive with *Staph. aureus* and all strains in phenon 4 were CAMP positive with *Rhod. equi*. None of the phenons could be equated with either *L. welshimeri* or *L. seeligeri*. Phenons 1 and 2 exhibited properties typical of *L. monocytogenes*. Phenon 3 contained the type strain of *L. innocua* and all were non-haemolytic. Phenon 4 exhibited the properties of *L. ivanovii*. Phenon 5 contained two rough, non-haemolytic strains received as *L. monocytogenes* and these could not be distinguished from the other strains in this phenon which were also non-haemolytic and rough. The five ungrouped strains that fell between phenons 3 and 4 were non-haemolytic even by the modifications of the CAMP test (Rocourt *et al.*, 1983b). In the absence of the genomic data for the type strains (Rocourt *et al.*, 1982) that were included in the study, there would be a strong argument for grouping all the strains in the phenons 1-5 and the related ungrouped strains into one phenon of species rank, and regarding the apparent clusters as subspecies. Such an interpretation is in agreement with electrophoretic protein patterns of strains of all the named species in the genus *Listeria* (Lamont *et al.*, 1986). These authors detected no significant interspecies variation between *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri* and *L. ivanovii*. There is virtually no correlation between species and serovar (Table 1) with the exception of *L. ivanovii* strains (phenon 4), all of which are members of serovar 5. The close phenotypic similarity between the five genomic species of *Listeria* has also been noted by McLauchlin (1987).

Listeria has always been recognized as an animal pathogen and in the last ten years, increasingly as a human pathogen (see McLauchlin, 1987). A comparative study (Rocourt *et al.*, 1983a) of the virulence to mice of the five genomic species of *L. monocytogenes sensu lato* (Rocourt *et al.*, 1982) showed that, under the conditions of test used, only *L. monocytogenes* and *L. ivanovii* were significantly pathogenic. *L. seeligeri* has also been isolated from a human infection (Rocourt *et al.*, 1986) and *L. innocua* has been recovered from human material (Rocourt & Seeliger, 1985). *L. welshimeri* has, to date, not been isolated from human sources (Rocourt &

Seeliger, 1985). Although the three species considered to be the most likely to cause listeriosis, *L. monocytogenes*, *L. seeligeri* and *L. ivanovii*, are all haemolytic under appropriate test conditions, the basis of virulence in *Listeria* species is still not understood. Because the five genomic species of *L. monocytogenes sensu lato* have such similar phenotypes, the presence of one species in a particular environment should be taken as indicative of the presence of the others. It is therefore important to distinguish these forms from the subspecies of *L. grayi* and from other similar bacteria such as *B. thermosphacta* and the atypical lactobacilli of phenons 7, 9, 10 and 11 which are also commonly found in raw and processed meat. The distinguishing characters of *L. monocytogenes sensu lato*, *L. grayi*, *B. thermosphacta* and the atypical lactobacilli are given in Table 2.

In conclusion the results of the study indicate the following. (1) The genomic species *L. monocytogenes*, *L. innocua* and *L. ivanovii* are phenotypically very similar but quite distinct from *L. grayi*. (2) *L. grayi* and *L. murrayi* represent only one species and on grounds of priority the species should be named *L. grayi*. (3) The results obtained with *L. denitrificans* are in agreement with the transfer of this bacterium to a new genus *Jonesia* (Rocourt *et al.*, 1987a). (4) Some strains designated group 1 by Thornley & Sharpe (1959) are members of the species *B. thermosphacta*. (5) Strains of groups 2 and 3 of Thornley & Sharpe (1959) represent four taxa not related to the genera *Brochothrix*, *Erysipelothrix* and *Listeria*. Phenotypically, they most closely resemble the genus *Lactobacillus* but further work is required to show whether they are members of this genus or an as yet undescribed taxon. (6) Some strains of lipophilic corynebacteria may be misidentified as *Erysipelothrix* if cultured on unsuitable media.

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