A Numerical Taxonomic Survey of *Listeria* and Related Bacteria

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SUMMARY

A numerical taxonomic study was performed on named strains of *Listeria*, *Erysipelothrix*, *Microbacterium thermosphactum*, *Lactobacillus*, *Streptococcus*, *Propionibacterium*, *Kurthia* and some possibly related bacteria using 143 unit characters covering a wide range of properties. The strains fell into six main clusters: (A) *Listeria*; (B) *Microbacterium thermosphactum*, *Lactobacillus*, *Streptococcus*; (C) *Gemella*, *Erysipelothrix*; (D) *Kurthia* and mainly aerobic corynebacteria; (E) *Propionibacterium*, *Staphylococcus*; (F) mainly *Cellulomonas*.

The genus *Listeria* contained three subgroups corresponding to (1) *Listeria monocytogenes*, (2) *Listeria grayi* and (3) non-haemolytic *Listeria* strains. *Listeria murrayi* did not appear to be sufficiently distinct from *Listeria grayi* to warrant separate species status. The genus *Erysipelothrix* was quite separate from the genus *Listeria*. *Microbacterium thermosphactum* was related to both the genera *Listeria* and *Lactobacillus* but formed a separate phenon that could appropriately be given generic rank. There were four distinct subgroups amongst the streptococci examined. *Gemella* strains appeared as a distinct phenon related to *Erysipelothrix* and the streptococci. The lactobacilli grouped into four phenons largely corresponding to the subgenera *Betabacterium*, *Streptobacterium* and *Thermobacterium* and to the species *Lactobacillus mali*.

Clusters A, B, and C displayed a relatively close association to each other and it is recommended that all be included in the family Lactobacillaceae.

INTRODUCTION

The genus *Listeria* was created to accommodate *Listeria monocytogenes*, first described by Murray, Webb & Swann (1926) as the causative organism of a septicaemic disease of rabbits. For many years a monospecific genus, *Listeria* now contains the additional species *List. denitrificans*, *List. grayi* and *List. murrayi* (Buchanan & Gibbons, 1974). However, the taxonomic relationships within the genus and between it and allied Gram-positive taxa are not clear. The genus *Listeria* was classified in the family Corynebacteriaceae (Breed, Murray & Smith, 1957), but in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974) it is treated with *Erysipelothrix* and *Caryophanon* as 'genera of uncertain affiliation' in a section which also contains lactobacilli.

The early literature on *List. monocytogenes* is well documented by Seeliger (1961) and more recent work (Errebo Larsen & Seeliger, 1966; Welshimer & Meredith, 1971; Stuart & Welshimer, 1973, 1974; Jones, 1975a; Wilkinson & Jones, 1975) indicates a close relationship between *List. monocytogenes*, *List. grayi* and *List. murrayi*, but there is disagreement on whether the relationship between the three taxa is consistent with three species in one genus (see Jones, 1975b). Recent studies indicate that *List. denitrificans* should be removed from

the genus *Listeria* (Stuart & Pease, 1972; Stuart & Welshimer, 1973, 1974; Jones, 1975a; Wilkinson & Jones, 1975).

Most numerical taxonomic studies suggest that *List. monocytogenes* is more closely related to the lactic acid bacteria than to the coryneform bacteria (Sneath & Cowan, 1958; Davis *et al.*, 1969; Stuart & Pease, 1972; Wilkinson, 1973; Jones, 1975a), though some also indicate a relationship between *List. monocytogenes* and the genus *Kurthia* and some animal corynebacteria (Da Silva & Holt, 1965; Davis & Newton, 1969).

In the present study, representatives of all the species of the genus *Listeria* and of the genera *Corynebacterium*, *Kurthia*, *Erysipelothrix*, *Lactobacillus*, *Streptococcus*, *Gemella*, *Staphylococcus*, *Micrococcus* and other Gram-positive bacteria received as '*Brevibacterium*', '*Arthrobacter*' and '*Bacterium eurydice*' were examined by numerical taxonomic methods. The '*Arthrobacter*' strains were isolated from clinical material and were included because a previous study (Jones, 1975a) suggested a possible relationship between these strains and *List. denitrificans*.

METHODS

Strains. These are listed in Table 1. A few were fresh isolates, but the majority were received from other workers or from culture collections. Type strains were included if possible. To encourage objectivity during scoring of the results, each strain was handled unnamed under a study number (G). Non-haemolytic variants of two *List. monocytogenes* strains (G15 and G16) which appeared during preliminary culture were retained in the study as G15A and G16A. All bacterial strains were lyophilized and working cultures were maintained on BM medium (see below).

Basal medium. In numerical taxonomic work it is important to select one medium which allows good growth of all the bacteria and can be used alone or with slight modifications as the basal medium for most of the tests. Of a number of basal media investigated, the most suitable was the MRS medium of De Man, Rogosa & Sharpe (1960) with acetate and citrate omitted. This modified MRS medium was used as a solid and liquid medium and is subsequently referred to as BM agar and BM broth. The bacteria used for the inoculation of all tests were 24 h cultures grown on BM agar or broth.

Choice of incubation temperature. *Microbacterium thermosphactum* will not grow at 35 °C whereas certain lactobacilli and streptococci grow weakly or not at all at 30 °C. Since the majority of the strains grew well at 35 °C, this was chosen as the main incubation temperature. *Microbacterium thermosphactum* and a representative selection of 46 strains from other genera were incubated at 30 °C. The representative strains were also grown at 35 °C so that any variation produced by incubating at different temperatures and the effect this might have on the subsequent clustering of the bacteria could be determined.

Colonial and cell morphology. Bacteria were grown on blood agar base (Oxoid) plus 7% (v/v) sterile horse blood. Haemolysis (α or β) was recorded at 1 and 2 days. Colonial morphology was examined under a low power microscope at 1 and 4 days at $\times 100$ and $\times 400$ magnification. Gram stains were performed (see Collins & Lyne, 1970) at the same time and examined at $\times 1000$ magnification.

Growth characteristics in liquid medium were examined in BM broth at 1 and 4 days. Motility was examined by the hanging drop technique at $\times 400$ magnification using BM broth cultures incubated for 1 day.

Growth at different temperatures. Bacteria were incubated in tubes of BM broth in water baths maintained at 5, 10, 20, 30, 35, 45 and 50 °C ± 1 °C. Growth was recorded daily for up to 21 days at 5 °C, 14 days at 10 and 20 °C, and up to 7 days at all other temperatures.

Table 1. *Strains studied*

Study no.	Name as received	Source and collection no.*	Origin or comments
G201	<i>Arthrobacter</i> sp.	Hill A137/68	Throat
G202	<i>Arthrobacter</i> sp.	Brzin	Throat
G172	<i>Bacterium eurydice</i>	Bailey	Honey bee
G173	<i>Bact. eurydice</i>	Bailey	Honey bee
G174	<i>Bact. eurydice</i>	Bailey	Honey bee
G164	<i>Brevibacterium acetyllicum</i>	ATCC953	Co-type†
G170	<i>Brev. ammoniagenes</i>	NCIB8143	Co-type†
G171	<i>Brev. divaricatum</i>	NCIB9397	—
G169	<i>Brev. imperiale</i>	ATCC8365	Holotype†
G165	<i>Brev. linens</i>	ATCC9174	Co-type
G167	<i>Brev. leucinophagum</i>	ATCC13809	Holotype†
G166	<i>Brev. stationis</i>	ATCC14403	—
G145	<i>Cellulomonas biazotea</i>	NCIB8077	Co-type†
			Same as NCIB8978
G148	<i>Cell. bibula</i>	NCIB8142	—
G146	<i>Cell. fimi</i>	NCIB8980	—
G147	<i>Cell. flavigena</i>	NCIB8073	Co-type†
G149	<i>Cell. rossica</i>	NCIB8074	—
G150	<i>Cell. uda</i>	ATCC491	Co-type†
G163	<i>Corynebacterium betae</i>	NCPBP363	—
G159	<i>Cor. diphtheriae</i>	NCTC3984	Suggested working type†
G155	<i>Cor. fascians</i>	NCPBP1488	—
G156	<i>Cor. insidiosum</i>	NCPBP83	—
G160	<i>Cor. manihot</i>	NCIB9097	—
G161	<i>Cor. mediolanum</i>	NCIB7206	—
G162	<i>Cor. michiganense</i>	NCPBP1468	—
G158	<i>Cor. poinsettiae</i>	NCPBP844	—
G157	<i>Cor. rathayi</i>	NCPBP797	—
G183	<i>Erysipelothrix rhusiopathiae</i>	Wellcome CN571, Woodbine	—
G184	<i>E. rhusiopathiae</i>	Wellcome CN354, Woodbine	—
G185	<i>E. rhusiopathiae</i>	Wellcome CN446, Woodbine	—
G186	<i>E. rhusiopathiae</i>	Wellcome CN906, Woodbine	—
G187	<i>E. rhusiopathiae</i>	Wellcome CN2432, Woodbine	—
G188	<i>E. rhusiopathiae</i>	Wellcome CN6260, Woodbine	—
G189	<i>E. rhusiopathiae</i>	S52, Woodbine	Pheasant, London Zoo
G190	<i>E. rhusiopathiae</i>	NCTC8163	Suggested working type†
G192	<i>E. rhusiopathiae</i>	Sneath 13	Human
G193	<i>E. rhusiopathiae</i>	Sneath 49	Human
G194	<i>E. rhusiopathiae</i>	Sneath 19	Human
G195	<i>E. rhusiopathiae</i>	Sneath 45	Human
G82	<i>Gemella haemolysans</i>	NCTC10459	—
G83	<i>G. haemolysans</i>	NCTC10243	—
G84	<i>G. haemolysans</i>	NCTC10244	—
G168	<i>Kurthia zopfii</i>	NCTC404	Suggested working type†
G111	<i>K. zopfii</i>	Gardner 26	Hamburger
G112	<i>K. zopfii</i>	Gardner 33	Frozen minced pork
G113	<i>K. zopfii</i>	Gardner 38	Pork sausage
G114	<i>K. zopfii</i>	Gardner 31	Fat trimmings
G115	<i>K. zopfii</i>	Gardner 34	Rendered lard
G116	<i>K. zopfii</i>	Gardner 76	Pork sausage
G117	<i>K. zopfii</i>	Keddie K2	Milk
G118	<i>K. zopfii</i>	Keddie S8	Meat
G119	<i>K. zopfii</i>	Keddie S9	Meat
G60	<i>Lactobacillus acidophilus</i>	ATCC4356	Suggested working type†
G61	<i>Lact. acidophilus</i> (biotype 1)	ATCC19992	—
G62	<i>Lact. brevis</i>	ATCC14869	Co-type†
G63	<i>Lact. buchneri</i>	ATCC4005	Suggested working type†
G64	<i>Lact. bulgaricus</i>	ATCC11842	Suggested working type†
G65	<i>Lact. casei</i>	ATCC393	Co-type†
G66	<i>Lact. casei</i> var. <i>rhamnosus</i>	ATCC7469	—
G67	<i>Lact. cellobiosus</i>	ATCC11739	Co-type†

* Abbreviations: ATCC, American Type Culture Collection; NCDO, National Collection of Dairy Organisms; NCIB, National Collection of Industrial Bacteria; NCPBP, National Collection of Plant Pathogenic Bacteria; NCTC, National Collection of Type Cultures; P.I.C., Pasteur Institute Collection; Bailey, L., Rothamstead Experimental Station, Harpenden, Hertfordshire; Board, R. G., Dept of Microbiology, University of Bath; Brzin, B., St Vincent's Hospital, Adelaide, Australia; Carr, J. G., Long Ashton Research Station, Bristol; Elliott, S. D., Dept of Pathology, University of Cambridge; Gardner, G. A., Ulster Curers' Association, Belfast; Hill, L. R., Central Public Health Laboratory, Colindale, London NW9; Jones, D., Dept of Microbiology, University of Leicester; Keddie, R. M., Dept of Microbiology, University of Reading; Kellogg, D. S., Communicable Disease Center, Atlanta, Georgia, U.S.A.; Khan, M. A., Dept of Microbiology, University of Nottingham, Sutton Bonnington; Kitchell, A. G., Meat Research Institute, Langford, Bristol; Lord, A., Dept of Microbiology, University of Leicester; Mair, N. S., Public Health Laboratory, Leicester; Murray, R. G. E., Dept of Bacteriology, University of Western Ontario, Canada; Seeliger, H. P. R., Insititute for Hygiene & Microbiology, University of Würzburg, Germany; Sneath, P. H. A., Dept of Microbiology, University of Leicester; Wellcome, The Wellcome Foundation, Beckenham, Kent; Wood, D., Dept of Microbiology, University of Leicester; Woodbine, M., Dept of Microbiology, University of Nottingham, Sutton Bonnington.

† From Sneath & Skerman (1966).

Table 1 (*cont.*)

Study no.	Name as received	Source and collection no.*	Origin or comments
G81	<i>Lact. delbrueckii</i>	NCDO1744	—
G68	<i>Lact. delbrueckii</i>	ATCC9649	Suggested working type†
G69	<i>Lact. fermentum</i>	ATCC14931	—
G70	<i>Lact. fermentum</i>	ATCC14932	—
G71	<i>Lact. fermentum</i> II	ATCC23272	—
G72	<i>Lact. helveticus</i>	ATCC15009	—
G73	<i>Lact. jugurt</i>	ATCC521	—
G74	<i>Lact. jugurt</i>	ATCC7993	—
G75	<i>Lact. lactis</i>	ATCC12315	Co-type†
G76	<i>Lact. leichmannii</i>	ATCC4797	Suggested working type†
G142	<i>Lact. mali</i>	Carr 12	Cider
G143	<i>Lact. mali</i>	Carr 123	Cider
G144	<i>Lact. mali</i>	Carr 26	Cider
G77	<i>Lact. plantarum</i>	ATCC14917	Suggested working type†
G78	<i>Lact. salivarius</i> subsp. <i>salivarius</i>	ATCC11741	Holotype†
G79	<i>Lact. salivarius</i> subsp. <i>salicinius</i>	ATCC11742	Co-type†
G80	<i>Lactobacillus</i> sp.	NCDO204	—
G1	<i>Listeria denitrificans</i>	Seeliger L84/68	—
G43	<i>List. denitrificans</i>	P.I.C.55134, L26	Type strain
G2	<i>List. grayi</i>	Seeliger L56/66	—
G3	<i>List. grayi</i>	Seeliger W-L96/65	—
G42	<i>List. grayi</i>	Seeliger L332/64	Chinchilla
G47	<i>List. grayi</i>	ATCC25400	—
G5	<i>List. monocytogenes</i>	Murray 148	Wolf-coyote hybrid
G6	<i>List. monocytogenes</i>	Murray 149	Wolf-coyote hybrid
G7	<i>List. monocytogenes</i>	Murray 44	Ferret
G10	<i>List. monocytogenes</i>	Murray 104	Arctic fox
G11	<i>List. monocytogenes</i>	Murray 127	Wild snowshoe hare
G12	<i>List. monocytogenes</i>	Murray 128	Moose brain
G13	<i>List. monocytogenes</i>	Murray 133	Tick
G14	<i>List. monocytogenes</i>	Murray 134	Spring water
G15	<i>List. monocytogenes</i>	Murray 139	Trout
G15A	<i>List. monocytogenes</i>	Murray 139	Trout
G16	<i>List. monocytogenes</i>	Murray 140	Trout
G16A	<i>List. monocytogenes</i>	Murray 140	Trout
G17	<i>List. monocytogenes</i>	Murray 141	Mud
G18	<i>List. monocytogenes</i>	Mair	Human
G19	<i>List. monocytogenes</i>	Mair 28251	Human
G20	<i>List. monocytogenes</i>	Mair 17	Human
G21	<i>List. monocytogenes</i>	Mair 26431	Human
G22	<i>List. monocytogenes</i>	NCTC5348	—
G23	<i>List. monocytogenes</i>	NCTC10357	Holotype†
G24	<i>List. monocytogenes</i>	NCTC5105	—
G25	<i>List. monocytogenes</i>	NCTC4885	—
G26	<i>List. monocytogenes</i>	Seeliger L88/7	Rough strain from Pote
G27	<i>List. monocytogenes</i>	Mair 017	—
G28	<i>List. monocytogenes</i>	NCTC4885	—
G29	<i>List. monocytogenes</i>	NCTC5105	—
G30	<i>List. monocytogenes</i>	NCTC5214	—
G31	<i>List. monocytogenes</i>	NCTC5348	—
G32	<i>List. monocytogenes</i>	NCTC7973	Holotype†
G33	<i>List. monocytogenes</i>	NCTC4883	—
G34	<i>List. monocytogenes</i>	Wood	Calf
G35	<i>List. monocytogenes</i>	Brzin	Human
G36	<i>List. monocytogenes</i>	Mair	Human
G37	<i>List. monocytogenes</i>	Jones	Calf brain
G38	<i>List. monocytogenes</i>	Khan 560	—
G39	<i>List. monocytogenes</i>	Khan 611	—
G40	<i>List. monocytogenes</i>	Khan 611	Non-haemolytic
G44	<i>List. murrayi</i>	ATCC25401	—
G45	<i>List. murrayi</i>	ATCC25402	—
G46	<i>List. murrayi</i>	ATCC25403	—
G4	<i>Listeria</i> sp.	Mair 24733	Human
G8	<i>Listeria</i> sp.	Murray 50	Cow brain
G9	<i>Listeria</i> sp.	Murray 51	Human tonsil
G191	<i>Listeria</i> sp.	Sneath 155	Human
G90	<i>Microbacterium thermosphactum</i>	Kitchell 1A (BaA1)	Beef
G91	<i>Micro. thermosphactum</i>	Kitchell 124 (BaA6)	Beef
G92	<i>Micro. thermosphactum</i>	Kitchell 13 (BaV3)	Beef
G93	<i>Micro. thermosphactum</i>	Kitchell 13A (BaV3)	Beef
G94	<i>Micro. thermosphactum</i>	Kitchell 13B (BaV3)	Beef
G95	<i>Micro. thermosphactum</i>	Kitchell 144 (BfA9)	Beef
G96	<i>Micro. thermosphactum</i>	Kitchell 156 (BfV9)	Beef
G97	<i>Micro. thermosphactum</i>	Kitchell 101 (LfA3)	Lamb

For footnotes see p. 401.

Table 1 (cont.)

Study no.	Name as received	Source and collection no.*	Origin or comments
G98	<i>Micro. thermosphactum</i>	Kitchell 46 (LfV1)	Lamb
G99	<i>Micro. thermosphactum</i>	Kitchell 128 (LfV6)	Lamb
G100	<i>Micro. thermosphactum</i>	Kitchell WR7	—
G101	<i>Micro. thermosphactum</i>	Kitchell CR12a	—
G102	<i>Micro. thermosphactum</i>	Kitchell 30	—
G103	<i>Micro. thermosphactum</i>	Kitchell 30a	—
G104	<i>Micro. thermosphactum</i>	NCIB10018	—
G86	<i>Micro. thermosphactum</i>	Lord 1	Prepacked pork
G87	<i>Micro. thermosphactum</i>	Lord 2	Prepacked pork
G88	<i>Micro. thermosphactum</i>	Lord 3	Prepacked pork
G89	<i>Micro. thermosphactum</i>	Lord 11	Prepacked pork
G105	<i>Micro. thermosphactum</i>	Lord 33	Prepacked pork
G106	<i>Micro. thermosphactum</i>	Gardner 44	—
G107	<i>Micro. thermosphactum</i>	Gardner 47	—
G108	<i>Micro. thermosphactum</i>	Gardner 52	—
G109	<i>Micro. thermosphactum</i>	Gardner 54	—
G110	<i>Micro. thermosphactum</i>	Gardner 58	—
G181	<i>Micrococcus agilis</i>	NCTC2676	Co-type†
G182	<i>Micrococcus denitrificans</i>	NCIB8944	Co-type†
G180	<i>Micrococcus hyicus</i>	NCTC10350	Co-type†
G179	<i>Micrococcus luteus</i>	NCTC8512	Proposed neotype†
G85	<i>Neisseria haemolysans</i>	Kellogg	—
G152	<i>Propionibacterium freudenreichii</i>	NCIB5959	—
G154	<i>Prop. pentosaceum</i>	NCIB8070	Suggested working type†
G153	<i>Prop. petersonii</i>	NCIB5962	Suggested working type†
G151	<i>Prop. shermanii</i>	NCIB8099	Suggested working type†
G177	<i>Staphylococcus aureus</i>	NCTC8532	Proposed neotype†
G178	<i>Staph. aureus</i>	NCTC6571	'Oxford strain'
G175	<i>Staph. epidermidis</i>	NCTC7291	—
G176	<i>Staph. saprophyticus</i>	NCTC7292	—
G121	<i>Streptococcus agalactiae</i>	NCTC8181	Neotype†
G123	<i>Strep. bovis</i>	NCTC8177	—
G124	<i>Strep. durans</i>	NCTC8307	Co-type†
G122	<i>Strep. equi</i>	NCTC9682	—
G140	<i>Strep. equinus</i>	ATCC9812	—
G125	<i>Strep. faecalis</i>	NCTC775	Suggested working type†
G126	<i>Strep. faecium</i>	NCTC7171	Suggested working type†
G127	<i>Strep. faecalis</i> var. <i>liquefaciens</i>	NCTC8175	—
G128	<i>Strep. faecalis</i> var. <i>zymogenes</i>	NCTC8176	—
G120	<i>Strep. pyogenes</i>	NCTC8198	Suggested working type†
G141	<i>Strep. suis</i>	Elliott PM1	Pig
G196	<i>Strep. suis</i>	Elliott PM35	Pig
G197	<i>Strep. suis</i>	Elliott PM37	Pig
G198	<i>Strep. suis</i>	Elliott 596/70	—
G199	<i>Strep. suis</i>	Elliott 627/70	—
G200	<i>Strep. suis</i>	Elliott 34/70	—
G139	<i>Strep. uberis</i>	NCTC3858	Co-type†
G129	<i>Streptococcus</i> sp.	NCTC5385	Serological gp E
G130	<i>Streptococcus</i> sp.	NCTC5389	Serological gp F
G131	<i>Streptococcus</i> sp.	NCTC8547	Serological gp G
G132	<i>Streptococcus</i> sp.	NCTC7868	Serological gp H
G133 O	<i>Streptococcus</i> sp.	NCTC5338	Serological gp K, opaque variant
G133 T	<i>Streptococcus</i> sp.	NCTC5338	Serological gp K, transparent variant
G48	<i>Streptococcus</i> sp.	NCTC10238	Serological gp L
G135	<i>Streptococcus</i> sp.	NCTC7760	Serological gp M
G136	<i>Streptococcus</i> sp.	NCTC6681	Serological gp N
G137	<i>Streptococcus</i> sp.	NCTC8029	Serological gp O
G134	<i>Streptococcus</i> sp.	NCTC9938	Serological gp Q
G138	<i>Streptococcus</i> sp.	Board	Sausage

For footnotes see p. 401.

Survival at 60 °C for 15 min. Bacteria grown in BM broth for 18 h were placed in a water bath maintained at 60 ± 1 °C for 15 min, then immediately cooled and two drops from each tube were plated on BM agar. The plates were observed for growth at 1 and 2 days.

Anaerobic growth. Duplicate tubes of BM broth were inoculated: one was incubated in an anaerobic jar under hydrogen, the other in air. Growth was recorded after 5 days.

Catalase and oxidase. Bacterial growth on BM agar was used for these tests. H_2O_2

(20 vol.) was used to test for catalase; oxidase was tested for by the method of Klinge (1960). A positive result was scored if a purple colour developed within 30 s.

Production of acid from carbohydrates. A modification of BM medium was used containing (g l⁻¹): peptone (Oxoid), 15; yeast extract (Oxoid), 1; Tween 80 (Koch-Light), 1; K₂HPO₄, 2; MgSO₄·7H₂O, 0·2; MnSO₄·4H₂O, 0·05; agar (Oxoid no. 1), 10; pH 7·0. For liquid medium, the agar was omitted. These media are subsequently referred to as SBM agar and SBM broth. Filter-sterilized aqueous solutions of the appropriate carbohydrates were added to the sterilized SBM medium to give a final concentration of 1 % (w/v). The indicator was 2 % aqueous bromocresol purple (1·5 ml l⁻¹). The medium was dispensed into divided dishes, inoculated as described by Stevens (1969) and examined for acid production at 1, 2, 3 and 4 days.

Oxidative/fermentative utilization of glucose. The method of Hugh & Leifson (1953) was used except that the basal medium was SBM broth plus 1 % aqueous bromocresol purple (3 ml l⁻¹) and 0·4 % (w/v) agar (Oxoid no. 1). Tubes were examined at frequent intervals for up to 14 days.

Gas production from glucose. Tubes of SBM broth plus 1 % (w/v) filter-sterilized glucose and containing inverted Durham tubes were inoculated, incubated and inspected for the accumulation of gas in the Durham tube at 1, 2, 5 and 7 days.

Gluconate oxidation. Tubes of SBM broth plus 1 % (w/v) gluconate were inoculated and incubated for 8 days. A Clinitest tablet (Ames Co., Slough) was then added to 1 ml of the growth medium. An orange colour indicated the presence of a reducing sugar (i.e. glucose), a blue one its absence.

Production of slime from sucrose. A filter-sterilized solution of sucrose was added to sterile SBM agar to give a final concentration of 1 % (w/v). The medium was dispensed in divided dishes and inoculated as described by Stevens (1969). Growth was inspected for slime formation at 1 and 2 days.

Methyl red and Voges Proskauer (VP) tests. BM broth [with glucose reduced to 0·5 % (w/v) and K₂HPO₄ increased to 0·5 % (w/v)] was dispensed in 12·5 ml amounts in 25 ml screw-capped bottles. After incubation, portions of the growth medium were tested as described by Cowan & Steel (1965), Barritt's method being used for the VP reaction.

Litmus milk. The medium and method of Wheater (1955) were used. Cultures were examined at 1, 2, 5 and 9 days for reduction before clotting, acid production, alkali production, clotting and digestion.

Carbon sources. Growth in SBM broth and in SBM broth containing the substrate under test were compared at 5 days by absorbance readings in a Bausch & Lomb spectrophotometer at 540 nm. Positive results were scored only when growth in the medium containing substrate was at least twice that in the basal broth.

Ammonia from peptone. SBM medium containing bromocresol purple (0·003 %, w/v) was dispensed in divided dishes and inoculated as described by Stevens (1969). Plates were examined for the appearance of a deep purple colour at 1, 2, 5 and 7 days.

Sensitivity to antibiotics. Petri dishes containing BM medium were lawned with 0·1 ml of 48 h broth cultures by means of a sterile glass spreader. Antibiotics in the form of 'Sentest' discs (Evans Medical, Liverpool) were then placed on to the growth (4 per plate). The concentrations of antibiotics are listed by Johnson & Sneath (1973). Zones of total and partial inhibition and, in the case of sulphonamide, stimulation of growth were recorded after 24 h and 48 h.

Cellulose hydrolysis. Cellulolytic activity was investigated by inoculating strains into tubes of BM broth (minus glucose) containing strips of Whatman no. 1 filter paper, half the

length of which was above the level of the liquid. The tubes were examined at frequent intervals for up to 10 weeks. A positive result was recorded if the filter paper disintegrated completely at the liquid/air interface.

Casein hydrolysis. An equal volume of 1 % (w/v) skim milk (Marvel; Cadbury, Bournville, Birmingham) in distilled water, sterilized by autoclaving at 120 °C for 15 min, was added to sterile BM agar (2 × normal agar concentration) and dispensed in Petri dishes. Cultures were streaked to give one line of growth and examined for areas of clearing for up to 15 days.

Lecithinase. Sterile lecithin (Oxoid, SR47), to give a final concentration of 1 % (v/v), was added to cool, sterile BM agar (with glucose reduced to 0.1 %) and dispensed in Petri dishes. Cultures were streaked to give one line of growth, incubated and inspected for zones of clearing and/or precipitation after 1, 2 and 8 days.

Deoxyribonuclease and ribonuclease. Bacteria were grown on BM agar plus 0.08 % (w/v) CaCl_2 and 0.2 % (w/v) deoxyribonucleic acid or 0.2 % (w/v) ribonucleic acid, dispensed in Petri dishes. Cultures were streaked in one line of growth. After 6 days incubation they were examined for zones of clearing. In the case of doubtful positive results, the plates were flooded with 1 M-HCl and then inspected for zones of clearing.

Urease and hydrogen sulphide production. The medium consisted of 40 ml 50 % (w/v) aqueous urea solution (sterilized by filtration) added to 1 l of sterile molten BM agar (glucose reduced to 0.5 % (w/v), but with 5 ml 2 % (w/v) phenol red added as indicator). The medium was dispensed in 5 ml quantities in test-tubes, allowed to set as slopes and inoculated. Strips of lead acetate paper were then inserted between the cap and the tube. After incubation, the tubes were inspected for blackening of the lead acetate paper and for development of a red colour in the medium at 1, 4, 7, 11, 14 and 21 days.

Tween hydrolysis. Tween 20, 40, 60 and 80 (Koch-Light), at final concentrations of 1 % (v/v), were added to BM agar minus Tween 80 and with glucose at 1 % (w/v). After sterilization at 120 °C for 15 min, the medium was dispensed in Petri dishes and inoculated as described for casein hydrolysis. Zones of precipitation and of clearing were recorded at frequent intervals up to 21 days.

Tyrosine and xanthine hydrolysis. BM agar (minus glucose) containing 0.5 % (w/v) L-tyrosine or 0.5 % (w/v) xanthine was autoclaved at 120 °C for 15 min and dispensed into Petri dishes, care being taken to ensure even distribution of the substrate. Plates were inoculated as described for casein hydrolysis and inspected for zones of clearing at frequent intervals up to 21 days.

Phosphatase and sulphatase. A 1 % (w/v) aqueous solution of phenolphthalein diphosphate (Na salt: BDH) or phenolphthalein disulphate (K salt: Koch-Light), sterilized by filtration, was added to sterile molten BM agar to give a final concentration of 0.001 % (w/v). The medium was dispensed in Petri dishes and inoculated to give well-separated colonies. After 6 days, 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 colonies due to the free phenolphthalein reacting with the ammonia vapour.

Aesculin hydrolysis. The method of Sneath (1960) was used, but the medium was SBM broth supplemented with (g l⁻¹): sodium citrate, 1; aesculin, 1; ferric citrate scales, 0.05. The ferric citrate scales were dissolved by boiling in a small volume of water and added to the medium which was dispensed in 3 to 4 ml quantities in 6 ml bottles and sterilized at 120 °C for 15 min. After inoculation and incubation, blackening and loss of purple fluorescence were recorded at 1 and 5 days.

Arginine deamination. The method of Thornley (1960) was used except that SBM broth

was used as the basal medium. Tests were performed with and without the liquid paraffin layer and tubes were inspected for the appearance of pink colour after 2 and 5 days.

Hippurate hydrolysis. SBM broth containing 1 % (w/v) sodium hippurate and 0.1 % (w/v) glucose, and sterilized by autoclaving at 120 °C for 15 min, was dispensed in 10 ml quantities in 25 ml bottles, inoculated and incubated for 7 days. Each culture was then centrifuged and two 1 ml samples were removed; to one was added 1 ml 12 % (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and to the other 1 ml 50 % (v/v) H_2SO_4 (Davis, 1955). Both test methods were used because weak positive reactions are frequently only detected by the H_2SO_4 method.

Starch hydrolysis. A 10 % (w/v) aqueous starch solution was dissolved by heating and added to BM agar (minus glucose) to give a final concentration of 0.2 % (w/v). After sterilization at 120 °C for 15 min, the medium was dispensed into Petri dishes and inoculated as for casein hydrolysis. After 15 days incubation the dishes were flooded with iodine solution and examined for clearing under and around the growth.

Indole production from tryptone. The medium [containing (g l^{-1}): tryptone (Oxoid), 20; K_2HPO_4 , 2; glucose, 2; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.05; pH 7.2] was dispensed into test-tubes in 7 ml quantities. After inoculation, the broths were incubated for 16 days and tested by method 2 of Cowan & Steel (1965).

Nitrate reduction. Bacteria were inoculated into BM broth plus 0.1 % KNO_3 dispensed in 10 ml quantities in tubes containing an inverted Durham tube. The tubes were examined at 2 and 8 days for the presence of gas. At the same time, samples were removed and tested for the presence of nitrite as described by Wilson & Miles (1964). Negative tests were checked at 8 days by adding zinc dust to reduce residual nitrate to nitrite.

Sensitivity to nitrite. Bacteria were inoculated into BM broth plus 0.01 % KNO_2 dispensed in 5 ml quantities in test tubes; BM broth alone was also inoculated as a control. Growth was compared visually in the two media at 2 and 8 days.

Sodium chloride. Bacteria were inoculated into tubes containing 5 ml BM broth plus 6.5 or 10 % (w/v) NaCl and into tubes of BM broth alone. After incubation, the tubes were examined for growth at 1, 2 and 7 days.

Inhibition by potassium thiocyanate and thallos acetate. BM agar containing potassium thiocyanate (2.5, 3.75 or 4.5 %, w/v) or thallos acetate (0.01, 0.02 or 0.03 %, w/v) was dispensed in divided dishes and inoculated as described by Stevens (1969). Growth was compared with that on BM agar alone at 1 and 2 days.

Potassium tellurite. A 10 % (w/v) aqueous solution of potassium tellurite, sterilized by autoclaving at 120 °C for 15 min, was added to sterilized BM agar to give final concentrations of 0.01, 0.025 and 0.05 % (w/v). The medium was dispensed and inoculated as described for potassium thiocyanate. Growth and degree of blackening were compared with that on BM agar alone at 1 and 2 days.

Tetrazolium. A filter-sterilized aqueous solution of 2,3,5-triphenyltetrazolium chloride (10 %, w/v) was added to sterile SBM agar to give final concentrations of 0.01 and 0.1 % (w/v). The medium was dispensed and inoculated as described for potassium thiocyanate. Growth and reduction of tetrazolium were compared with that on SBM agar alone at 1 and 2 days.

Coding of characters. Those characters which existed in one or two mutually exclusive states were scored 1 (plus) and 0 (minus). Multistate characters, e.g. degree of acid production, were scored 0 (minus), 1 (weak positive) and 2 (strong positive).

Computation. The simple matching coefficient (S_{SM}) (Sokal & Michener, 1958) was used and clustering was achieved by both single linkage (Sneath, 1957) and unweighted pair-

group average linkage (Sokal & Michener, 1958). The IGROUPS (integer groups) program printed character-value statistics on specified groups of bacteria. This program, devised by M. J. Sackin of this Department, aided the selection of tests important in the characterization of taxonomic groups. A further program PRINTRES, written by B. J. Wilkinson and M. J. Sackin, printed out the raw data. All calculations were carried out on the ICL 4130 computer at Leicester University.

The maximum number of strains which could be computed at one time was 180, therefore thirteen of the 193 strains included in the study were omitted in the first computation (computation A). As mentioned above, 46 strains were tested for all characters at both 30 and 35 °C. These duplicate sets of results, together with a single set of results for a further 88 strains (including the strains omitted in the first computation), were computed separately (computation B). A further computation was carried out on those strains (area I of computation A) which showed the closest relationship to *Listeria* and the *K. zopfii* strains.

RESULTS

Computation A

All strains except *Micro. thermosphactum* (G86 to G97 inclusive) and *Micrococcus agilis* (G181) were included in the first computation. The *Micro. thermosphactum* strains included were incubated at 30 °C, the remainder of the strains at 35 °C.

At the 67 % similarity level (%S), all 180 strains fell into two main areas (I and II). At just below 75 % S, these areas contained six distinct clusters (A, B, C, D, E, F) with varying degrees of homogeneity. The quantitative taxonomic relationships within and between the clusters are illustrated in Fig. 1.

Cluster A: Listeria. Above 80 % S this cluster contained three subgroups and one single strain. Subgroup A1 with an internal similarity of 87 %, contained all strains received as *List. monocytogenes*, two received as *Listeria* sp., and one received as *List. denitrificans* (G1) which biochemically and serologically was typical of *List. monocytogenes*. Subgroup A2, with an internal similarity of 90 %, contained all strains received as *List. grayi* and *List. murrayi* and joined the type strain of *List. denitrificans* (G43) at approximately 85 % S. Subgroup A3 contained three non-haemolytic listeria strains.

Cluster B: Lactobacillus (in part), *Micro. thermosphactum*, *Propionibacterium*, *Streptococcus* (in part). This cluster comprised six subgroups. Subgroup B1, with an internal similarity of 80 %, contained strains of *Lact. casei*, *Lact. plantarum* and *Lact. salivarius* plus strains received as *E. rhusiopathiae* (G189) and as *Listeria* sp. (G9). Subgroup B2, with an internal similarity of 90 %, contained strains received as *Micro. thermosphactum*. Subgroup B3, with an internal similarity of 95 %, contained three strains of *Lact. mali*. Subgroup B4, containing all strains received as *Propionibacterium* spp., was only just included in cluster B at 75 % S (see Fig. 1). Subgroup B5 was a rather loose cluster containing streptococci of different serological groups. Subgroup B6 (internal similarity 82 %) contained all the streptococci of serological groups D and Q with the exception of *Strep. durans* which fell in subgroup B5. *Streptococcus uberis* was loosely associated with subgroups B5 and B6.

Cluster C: 'Bacterium eurydice', Erysipelothrix, Gemella, Lactobacillus (in part), *Streptococcus* (in part). Subgroups C1 (internal similarity 82 %) and C2 (internal similarity 80 %) corresponded in the main to the subgenera *Thermobacterium* and *Betabacterium* (Orla-Jensen, 1919; Rogosa & Sharpe, 1959). Subgroup C3, a small homogeneous cluster (internal similarity 90 %), contained four strains received as *G. haemolysans* and *N. haemolysans*. Subgroup C4 contained three strains of '*Bact. eurydice*' (internal similarity 90 %) and *Brev.*

divaricatum which joined the '*Bact. eurydice*' strains at 82 % S. Subgroup C5 was a small group containing three strains of streptococci, one of serological group F and two morphological variants of serological group K. Subgroup C6 contained 11 strains received as *E. rhusiopathiae*. Subgroup C7 contained five strains of *Strep. suis*. Subgroup C8 contained three strains of streptococci, one received as *Strep. suis*, one strain of serological group M and one strain of serological group O.

Cluster D: *Brevibacterium*, *Corynebacterium*, *Kurthia*, *Micrococcus*. At 75 % S this cluster comprised four subgroups. Subgroup D1 contained all strains received as *K. zopfii* and joined subgroup D2, containing *Brev. ammoniagenes*, *Brev. stationis* and *Micrococcus denitrificans*, at an S-level just below 80 %. Three strains received as *Cell. rossica*, *Cor. mediolanum* and *Brev. leucinophagum* joined subgroups D1 and D2 at about 77 % S. Subgroup D3 contained three strains, *Cell. fimi*, *Cor. fascians* and *Cor. rathayi*. A heterogeneous subgroup, D4 (80 % S), contained three plant pathogenic corynebacteria, *Brev. linens* and *Brev. imperiale*.

Cluster E: *Staphylococcus*, *Cor. insidiosum*. This was a small cluster containing six strains (internal similarity 84 %).

Cluster F: *Cellulomonas*, *Corynebacterium*. At 78 % this cluster comprised two subgroups: F1, containing *Cellulomonas* spp., *Cor. diphtheriae* and *Cor. manihot*, and F2 containing *Brev. acetylicum* and two strains received as '*Arthrobacter*' spp. isolated from the human throat.

Table 2. Characteristics of some subgroups in computation C (Fig. 3)
as percentages of positive reactions

	<i>List. monocytogenes</i> (A1)	<i>List. grayi</i> / <i>List. murrayi</i> (A2)	Non-haemolytic <i>Listeria</i> (A3)	<i>Micro. thermosphactum</i> (B2)	<i>Lactobacillus</i> (B1)	<i>Lact. mali</i> (B3)	<i>Lactobacillus</i> (C2)	<i>Lactobacillus</i> (C1)
No. of strains tested	37	7	3	25	6	3	7	9
Colonial characters:								
Smooth	97	100	66	100	83	0	86	89
Edge entire	22	14	0	4	17	0	29	0
Elevation low convex	100	100	0	8	33	100	29	0
Elevation convex	0	0	0	0	0	0	0	0
Elevation 'fried egg'	0	0	100	92	67	0	71	100
Consistency watery	97	100	100	50	83	66	71	11
Consistency butyrous	3	0	0	50	17	33	29	89
Translucent	100	43	100	16	83	0	100	100
Emulsification homogeneous	100	100	100	40	100	66	86	89
Adheres to medium	78	86	66	8	0	0	0	11
Diameter > 3 mm	0	0	0	16	0	0	0	0
β -Haemolysis	92	0	0	0	0	0	0	0
Slime production from sucrose	0	0	0	0	0	100	0	0
Stained preparations:								
Beaded Gram stain	0	0	0	100	100	100	100	100
Long rods > 2 μ m	0	0	0	44	50	100	100	100
Short rods < 2 μ m	100	100	100	56	50	0	0	0
Filaments	5	0	66	52	33	100	57	67

Table 2 (cont.)

	<i>List. monocytogenes</i> (A ₁)	<i>List. grayi/List. murrayi</i> (A ₂)	Non-haemolytic listeria (A ₃)	<i>Micro. thermosphactum</i> (B ₂)	<i>Lactobacillus</i> (B ₁)	<i>Lact. mali</i> (B ₃)	<i>Lactobacillus</i> (C ₂)	<i>Lactobacillus</i> (C ₁)
Stained preparations (cont.):								
Cells in chains	100	100	100	92	83	100	100	100
Pleomorphism	0	0	0	60	0	0	43	33
Pellet in liquid medium:								
Stringy	100	100	100	96	83	100	100	100
Granular	0	0	0	0	17	0	0	0
Floccular	0	0	0	4	0	0	0	0
Easily suspended	100	100	100	52	83	100	86	100
Motility	97	100	0	0	0	100	0	11
Tumbling motility	97	100	0	0	0	0	0	0
Growth at:								
5 °C	100	100	100	100	50	100	0	11
10 °C	100	100	100	100	67	100	43	11
35 °C	100	100	100	0	100	100	100	100
45 °C	54	100	0	0	100	0	57	78
50 °C	3	0	0	0	0	0	0	22
Anaerobic growth	97	100	100	100	100	100	100	100
Catalase	97	100	0	84	0	100	0	0
Acid from:								
Inulin	0	0	100	96	83	66	0	33
Inositol	0	0	100	100	67	0	0	22
Dulcitol	5	71	66	96	83	66	0	22
Lactose	95	100	100	100	100	100	86	78
Methyl α -glucoside	100	100	100	100	83	100	29	44
Melibiose	11	86	33	100	100	66	71	44
Salicin	100	100	100	100	100	100	0	33
Adonitol	46	100	33	96	50	100	0	11
L-Arabinose	0	14	33	0	17	0	43	0
Mannitol	8	100	100	88	100	0	14	0
Glycerol	76	100	100	100	83	0	0	11
Maltose	100	100	100	100	100	100	86	78
Rhamnose	95	29	33	0	50	100	0	0
D-Galactose	19	100	100	100	100	0	71	67
Sucrose	5	0	100	100	100	100	86	56
Sorbitol	0	14	66	100	100	0	0	0
Fructose	100	100	100	100	100	100	86	67
Xylose	30	100	66	96	17	100	29	0
Trehalose	95	100	100	100	100	100	14	33
Cellobiose	100	100	100	100	83	100	14	33
Melezitose	59	100	100	100	50	33	14	33
Raffinose	8	100	66	100	67	100	86	44
Gas from glucose	0	0	0	0	0	0	86	0
Gluconate oxidation	0	0	0	0	17	0	14	0
Degradation tests:								
Aesculin	100	100	100	100	83	100	14	11
Arginine (aerobic)	3	0	0	0	0	0	0	0
Arginine (anaerobic)	0	0	33	0	0	0	86	0
Hippurate	100	43	100	0	50	66	14	0
Lecithinase	84	86	33	52	100	100	57	89

Table 2 (cont.)

	<i>List. monocytogenes</i> (A1)	<i>List. grayi</i> / <i>List. murrayi</i> (A2)	Non-haemolytic <i>Listeria</i> (A3)	<i>Micro. thermosphactum</i> (B2)	<i>Lactobacillus</i> (B1)	<i>Lact. mali</i> (B3)	<i>Lactobacillus</i> (C2)	<i>Lactobacillus</i> (C1)
Degradation tests (cont.):								
DNA	100	100	100	100	50	33	57	100
RNA	100	100	100	100	33	0	43	89
Starch	8	71	0	24	100	0	100	100
Tween 40	0	0	0	8	17	0	14	11
Tween 60	89	0	0	92	100	100	57	33
Tween 80	100	0	0	0	0	0	0	11
Carbon sources:								
Pyruvate	3	0	0	0	0	0	29	0
Acetate	3	0	0	0	17	0	0	0
Propionate	3	0	0	0	0	0	0	11
Citrate	3	0	0	0	0	0	14	11
Malate	89	29	0	0	17	0	0	0
Malonate	0	14	0	0	33	0	14	11
α -Ketoglutarate	3	0	0	0	0	0	0	0
Salicylate	3	0	0	0	33	0	0	0
Growth in the presence of:								
NaCl								
6.5% (w/v)	100	100	100	100	100	66	71	11
10.0% (w/v)	97	100	100	92	33	66	43	11
Potassium nitrite								
0.1% (w/v)	95	86	100	0	0	0	0	0
Sodium azide								
0.01% (w/v)	16	100	66	4	100	100	100	100
0.02% (w/v)	8	100	33	0	100	0	100	100
0.05% (w/v)	3	14	0	0	100	0	100	78
Potassium thiocyanate								
2.5% (w/v)	100	100	100	84	100	100	100	44
3.75% (w/v)	100	100	100	0	100	100	100	22
4.5% (w/v)	100	100	100	0	50	100	86	22
Tetrazolium								
0.01% (w/v)	100	100	100	100	100	100	100	100
Reduction 0.01% (w/v)	100	100	100	100	83	0	100	78
0.1% (w/v)	97	100	100	48	67	100	86	56
Reduction 0.1% (w/v)	100	100	100	32	33	0	86	33
Reduction of potassium tellurite								
0.01% (w/v)	100	100	100	96	83	100	80	33
0.025% (w/v)	100	100	100	68	33	66	80	11
0.05% (w/v)	100	100	100	88	33	33	80	22
Sensitivity to:								
Penicillin	100	100	100	100	100	66	100	100
Streptomycin	100	100	100	4	83	100	100	100
Sulphanilamide	3	0	0	64	0	0	14	0
Novobiocin	100	100	66	100	100	100	100	100
Nalidixic acid	0	0	0	100	0	0	0	0
Stimulation of growth by sulphanilamide	68	43	0	0	0	33	14	11
Voges Proskauer	100	100	100	88	100	0	0	11
Reduction NO ₃ → NO ₂	0	43	0	0	0	0	0	0
Production of H ₂ S	0	57	0	0	83	100	43	56
Urease	0	0	33	0	0	0	0	0
Phosphatase	100	100	66	100	100	100	86	33

All strains were Gram-positive; grew at 20 and 30 °C; fermented glucose and utilized it as sole carbon source; grew in the presence of 0.01%, 0.02%, 0.03% (w/v) thallous acetate; were indole and sulphatase negative; and were sensitive to chloramphenicol, chlortetracycline, erythromycin, oleandomycin, oxytetracycline and tetracycline. None survived heating at 60 °C for 15 min; nor produced NH₃ from peptone; nor degraded casein, cellulose, Tween 20, tyrosine or xanthine.

Table 3. Characteristics of some subgroups in computation C (Fig. 3*)
as percentages of positive reactions

	<i>Streptococcus</i> (B5)	<i>Streptococcus</i> (B6)	<i>Streptococcus</i> (C8)	<i>Streptococcus</i> (C7)	<i>Gemella</i> (C3)	<i>Erysipelothrix</i> (C6)	<i>Kurthia zopfii</i> (D1)
No. of strains tested	11	8	4	5	4	11	9
Colonial characters:							
Smooth	0	100	100	100	100	100	56
Edge entire	91	100	100	0	25	0	0
Edge rhizoidal	0	0	0	0	0	0	100
Elevation low convex	91	100	100	0	25	18	67
Elevation 'fried egg'	9	0	0	100	75	82	33
Consistency watery	82	100	100	20	50	55	0
Consistency butyrous	18	0	0	80	50	45	100
Translucent	82	75	75	100	25	82	0
Emulsification homogeneous	27	50	75	100	100	100	33
Adheres to medium	45	63	0	80	0	100	22
Diameter > 3 mm	0	0	0	0	0	0	100
Pigment yellow	0	0	0	0	0	0	100
β -Haemolysis	82	13	50	100	0	0	0
α -Haemolysis	9	88	25	0	0	0	0
Slime production from sucrose	0	0	25	0	0	0	0
Stained preparations:							
Gram positive	91	100	100	100	0	100	89
Beaded Gram stain	0	0	0	0	0	0	22
Long rods > 2 μ m	0	0	0	0	0	0	100
Short rods < 2 μ m	0	0	0	0	0	100	0
Filaments	0	0	0	0	0	0	44
Cocci	100	100	100	100	100	0	0
Pleomorphism	0	0	0	0	25	0	0
Pellet in liquid medium:							
Stringy	91	100	100	100	50	100	89
Granular	0	0	0	0	50	0	11
Floccular	9	0	0	0	0	0	0
Easily suspended	64	88	75	100	100	27	56
Motility	0	100	0	0	0	0	67
Growth at:							
5 °C	18	75	0	0	0	18	66
10 °C	18	88	25	100	100	100	100
45 °C	18	88	25	0	0	0	44
50 °C	9	13	0	0	0	0	0
Survival at 60 °C for 15 min	0	60	0	0	0	0	0
Anaerobic growth	100	100	100	100	100	100	0
Catalase	9	25	25	0	0	0	100
Acid from:							
Inulin	45	100	50	80	0	0	0
Inositol	18	63	0	0	0	0	0
Dulcitol	27	75	0	0	0	0	0
Lactose	82	100	100	100	0	100	0
Methyl α -glucoside	55	100	0	100	0	0	0
Melibiose	36	100	0	100	0	0	0
Salicin	73	100	25	100	0	0	0
Adonitol	36	100	0	0	0	0	0
L-Arabinose	18	50	0	0	0	0	0
Mannitol	18	75	25	0	0	0	0
Glycerol	18	63	0	0	0	0	67

Table 3 (cont.)

	<i>Streptococcus</i> (B5)	<i>Streptococcus</i> (B6)	<i>Streptococcus</i> (C8)	<i>Streptococcus</i> (C7)	<i>Gemella</i> (C3)	<i>Erysipelothrix</i> (C6)	<i>Kurthia zopfii</i> (D1)
Acid from (cont.):							
Maltose	100	100	100	100	100	100	0
Rhamnose	9	50	25	0	0	0	0
D-Galactose	100	100	50	100	0	100	0
Sucrose	82	100	75	100	100	27	0
Sorbitol	9	63	25	0	0	0	0
Fructose	100	100	75	100	100	100	22
Xylose	27	63	50	80	50	9	0
Trehalose	64	88	50	100	0	18	0
Cellobiose	73	100	50	100	0	91	0
Melezitose	9	88	0	80	0	0	0
Raffinose	55	100	50	100	0	0	0
Glucose							
Oxidative	0	0	0	0	0	0	0
Fermentative	100	100	100	100	100	100	0
Degradation tests:							
Aesculin	64	100	75	100	0	0	0
Arginine (aerobic)	0	0	0	0	0	0	100
Arginine (anaerobic)	82	75	75	100	0	100	0
Hippurate	18	75	25	20	0	55	67
Lecithinase	73	88	0	100	100	82	100
DNA	100	100	50	0	100	0	44
RNA	100	75	25	0	100	0	22
Starch	45	13	25	100	0	0	0
Tween 20	0	0	0	0	0	0	89
Tween 40	9	0	0	20	0	0	22
Tween 60	91	63	75	0	100	0	22
Tween 80	0	0	0	20	0	0	100
Tyrosine	9	50	0	0	0	0	0
Carbon sources:							
Glucose	100	100	100	100	100	100	44
Citrate	0	0	25	0	0	0	11
Malate	64	88	75	20	100	9	22
α -Ketoglutarate	0	0	25	0	0	0	0
Salicylate	0	50	0	0	0	18	11
Growth in the presence of:							
NaCl							
6.5 % (w/v)	91	100	50	100	100	82	100
10.0 % (w/v)	55	100	50	80	100	64	100
Potassium nitrite							
0.1 % (w/v)	9	0	50	100	0	0	89
Thallous acetate							
0.02 % (w/v)	100	100	100	100	100	100	89
0.03 % (w/v)	100	100	100	100	100	100	89
Sodium azide							
0.01 % (w/v)	100	100	75	20	100	91	0
0.02 % (w/v)	100	100	25	0	100	91	0
0.05 % (w/v)	45	100	0	0	100	0	0
Potassium thiocyanate							
2.5 % (w/v)	36	88	0	0	100	0	100
3.75 % (w/v)	9	88	0	0	100	0	100
4.5 % (w/v)	0	50	0	0	100	0	67

Table 3 (cont.)

	<i>Streptococcus</i> (B5)	<i>Streptococcus</i> (B6)	<i>Streptococcus</i> (C8)	<i>Streptococcus</i> (C7)	<i>Gemella</i> (C3)	<i>Erysipelothrix</i> (C6)	<i>Kurthia zopfii</i> (D1)
Growth in the presence of (cont.):							
Tetrazolium							
0.1 % (w/v)	82	88	25	0	100	100	89
Reduction 0.1 % (w/v)	82	88	25	0	100	100	89
Reduction of potassium tellurite							
0.01 % (w/v)	100	100	0	20	0	91	100
0.025 % (w/v)	91	100	0	0	0	91	100
0.05 % (w/v)	73	75	25	100	0	73	78
Sensitivity to:							
Penicillin	100	88	100	100	100	100	100
Sulphanilamide	45	0	25	60	75	27	22
Novobiocin	100	100	100	100	100	0	100
Nalidixic acid	9	25	0	0	50	82	89
Methyl red	100	100	100	100	100	100	0
Voges Proskauer	9	75	25	80	50	0	0
Ammonia from peptone	0	0	0	0	0	0	100
Reduction $\text{NO}_3 \rightarrow \text{N}_2$	0	0	0	0	0	0	11
Production of H_2S	55	25	25	0	75	64	0
Urease	0	0	0	0	0	0	100
Phosphatase	100	75	25	0	100	55	100

All strains formed chains; grew at 20, 30 and 35 °C; grew in presence of 0.01 % (w/v) thallos acetate and grew on and reduced 0.01 % (w/v) tetrazolium; were indole, oxidase and sulphatase negative; and were sensitive to chloramphenicol, chlortetracycline, erythromycin, oleandomycin, oxytetracycline, streptomycin and tetracycline. No strains showed tumbling motility; produced gas from glucose; degraded casein, cellulose or xanthine; utilized acetate, malonate, propionate or pyruvate as carbon sources; reduced NO_3 to NO_2 ; nor oxidized gluconate.

* *Kurthia zopfii* strains are not included in Fig. 3 because they are too distantly related to the other taxa.

Computation B

This computation (Fig. 2) included the 13 strains omitted from computation A, and the two sets of results obtained with 46 representative strains tested at 30 and 35 °C. To accommodate these extra strains and results, 59 strains included in computation A were omitted.

There was reasonably good accord between the relationships of the strains in the two computations. The composition of cluster A was the same in both. So, in the main, was cluster B, but subgroup B4 (*Propionibacterium*) moved to join cluster E (Fig. 1) containing staphylococci and *Cor. insidiosum* at 77 % S. In cluster C, subgroup C5 [streptococci of serological groups F and K (Fig. 1)] merged with the large heterogeneous streptococcal subgroup B5. Also, whereas in cluster C (Fig. 1), *Lact. acidophilus* (G61) showed a relationship to other lactobacilli in subgroup C2, in computation B (Fig. 2) it moved to show a low level of relationship to some streptococci in subgroup C8. The relationship between strains in clusters D and F was not markedly affected despite the fact that far fewer strains from these clusters were included in computation B.

Fig. 3. Model depicting approximate spatial relationships between the taxa (except *Kurthia*) included in computation C.

Computation C

This computation was performed on 140 strains from area I (Figs 1 and 2) representing the taxa *Erysipelothrix*, *Gemella*, *Lactobacillus*, *Listeria*, *Microbacterium thermosphactum*, *Streptococcus*, and nine strains of *K. zopfii*. The data were further analysed by the IGROUPS and PRINTRES programs to determine the tests of importance in the characterization of groups in this area. A model (Fig. 3) showing the approximate spatial relationships of the inter- and intra-group similarities in area I was constructed from this data. Tables 2 and 3 list the test results characteristic of the groupings shown in Fig. 3, plus *K. zopfii* (Table 3).

Reproducibility

Reproducibility of microbiological tests is receiving increasing attention (Sneath & Collins, 1974). The effect of such errors on bacterial classification has been discussed by Sneath & Johnson (1972).

In the present study all the tests were repeated on approximately 25 % of the strains and 46 strains were subjected to the same battery of tests incubated at 30 and 35 °C. In addition five strains were divided into two and treated as separate cultures in all tests.

The five pairs of duplicate cultures showed a similarity between 88.5 % and 92.7 % to their respective partners giving an average discrepancy of 9 %. A comparison of the two sets of data obtained on the same strains cultured at 30 and 35 °C showed a discrepancy of 11 %. The data obtained from the tests performed on the same strains at the same temperature, but at different times, showed an average error of about 15 %.

This last figure is high compared with earlier studies (Lapage *et al.*, 1970; Sneath & Johnson, 1972; Jones, 1975a). An analysis of the results showed that the error was due to the results of morphological tests, where interpretation is largely subjective, and to the results of tests such as growth at various temperatures, where slight changes in conditions of incubation can have a marked effect on the results. However, the duplicate tests were only performed on 25 % of the strains (see above) and we are of the opinion that the effect of this test error on the clustering of the bacteria was less than the information lost by the omission of these results for all the strains (for discussion, see Sneath, 1972).

DISCUSSION

The grouping of the strains into two main areas, one containing *Erysipelothrix*, *Gemella*, *Lactobacillus*, *Listeria*, *Micro. thermosphactum*, *Propionibacterium* and *Streptococcus*, the other *Brevibacterium*, *Corynebacterium*, *Kurthia*, *Micrococcus* (Figs 1 and 2), agrees with the results of previous studies which suggested that the genus *Listeria* is more closely related to the lactic acid bacteria than to the coryneform bacteria (Davis *et al.*, 1969; Stuart & Pease, 1972; Jones, 1975*a*).

Area II contained those bacteria which were included mainly as marker organisms and thus, with the exception of the genus *Kurthia*, were represented only by single strains. The clustering of all the *K. zopfii* strains as a homogeneous group (85 % S), but with indications of two separate clusters within the taxon, is in keeping with earlier findings (Keddie, 1949; Gardner, 1969; Jones, 1975*a*) and the grouping of the marker strains is in good accord with the results of Bousfield (1972) and Jones (1975*a*).

The grouping in area I of the strains of the genus *Propionibacterium*, the type strain of *List. denitrificans* and three strains of '*Bact. eurydice*' was unexpected. The numerical taxonomic results of Seyfried (1968) indicated that the genera *Loctobacillus* and *Propionibacterium* were 'distinct in their relationship' and in a similar, but broader, study (Jones, 1975*a*), the genus *Propionibacterium* grouped as a distinct taxon more closely related to the corynebacteria than to the lactic acid bacteria. The % guanine + cytosine (GC) base ratio (57 to 68 %, Johnson & Cummins, 1972) is in keeping with the results of Jones (1975*a*). However, although the propionibacteria grouped in area I in the present study, the position of the taxon was not stable. In computation A it showed a relationship to *Lact. mali* (Fig. 1), while in computation B it formed a loose association with strains of *Staph. aureus* and *Cor. insidiosum* (Fig. 2). Little is yet known about the effect of the composition of the groups of bacteria studied on clustering at levels higher than that of genus. At these taxonomically higher levels, the 'distances' between genera may be roughly equal. Hence, just which way an intermediate genus (in this case *Propionibacterium*) will cluster at a higher level may depend on sampling error.

The clustering of the one strain of *List. denitrificans* (% GC 55; Stuart & Welshimer, 1973) in area I so close to other representatives of the genus *Listeria* (% GC 35; Stuart & Welshimer, 1973) is also not easy to explain. Neither is the discrepancy between the association of the three strains of '*Bact. eurydice*' with the genera *Gemella* and *Lactobacillus* in the present study and its apparent relationship to some corynebacteria in the study of Jones (1975*a*). However, Jones (1975*a*) noted that when the test results of two groups of strains (75 % of which were common to both groups) were computed, the main taxa remained constant in their relationship to each other, but poorly represented taxa or single strains, which were not the type strains of good taxa, moved. It must be accepted that single strains may not always represent taxa very well, but the remedy here is to conduct further studies on more strains of these poorly sampled species. The differences in the relationship of certain taxa to each other noted between studies carried out in different laboratories may be due as much to the number and type of bacteria studied as to the selection of tests and test reproducibility.

Intragenetic relationships of Listeria

All the strains received as *Listeria* species, with the exception of G9, group together at 83 % S (cluster A). Within this cluster the appearance of three subgroups corresponding to *List. monocytogenes* (A1), *List. grayi*/*List. murrayi* (A2), *Listeria* spp. (A3) and one strain of *List. denitrificans* (G43) is only in partial agreement with the results of previous workers

Table 4. Characters which differentiate between the three main taxa in the genus *Listeria* and *List. denitrificans*

	<i>List.</i> <i>monocytogenes</i>	<i>List.</i> <i>grayi</i>	<i>List.</i> and <i>murrayi</i>	Non-haemolytic <i>listeria</i> (A3)	<i>List.</i> <i>denitrificans</i>
β -Haemolysis	+	—	—	—	—
Motility	+	+	+	—	+
Catalase	+	+	+	—	+
Voges Proskauer	+	+	+	+	—
Nitrate reduction	—	—	+	—	+
Arginine hydrolysis	—	—	—	+	—
Cellulose hydrolysis	—	—	—	—	+
Ribonuclease	+	+	+	+	—
H ₂ S production	—	+	+	—	—
Acid production from:					
L-Arabinose	—	+	—	+	+
D-Galactose	+	+	+	+	+
Inositol	—	—	—	+	—
Inulin	—	—	—	+	—
Lactose	+	+	+	+	+
Mannitol	+	+	+	+	—
Raffinose	+	+	+	+	+
Rhamnose	+	—	+	+	—
Sucrose	+	—	—	+	+

+, Positive (figures in parentheses indicate % positive, otherwise all strains positive); —, negative.

(Stuart & Pease, 1972; Stuart & Welshimer, 1973, 1974; Jones, 1975*a*). The surprising association of *List. denitrificans* at 86 % S with subgroup A2 (*List. grayi*/*List. murrayi*) has been discussed above.

All previous studies have indicated the separate identity of *List. monocytogenes* and this has also been shown by our results. As was noted by Stuart & Pease (1972) and Wilkinson & Jones (1975), no correlation could be detected between serovars and physiological groupings within this species.

Strains of *List. grayi* and *List. murrayi* clustered together at 90 % S and although two distinct subgroupings can be seen they do not correlate with the names carried by the strains. Therefore the suggestion of Stuart & Welshimer (1973, 1974) that there are two distinct species, *List. grayi* and *List. murrayi*, is premature. *Listeria murrayi*, in our opinion, should be regarded as a biovar of *List. grayi*.

The recovery of subgroup A3 is interesting. Two of the three strains were isolated as non-haemolytic variants or contaminants from haemolytic cultures of *List. monocytogenes* (G15A and G16A) and the other (G8) was isolated as *List. monocytogenes* from the brain of a cow by the late E. G. D. Murray. Further work is required to clarify the taxonomic relationships of these non-haemolytic strains. Non-haemolytic strains of *List. monocytogenes* have been isolated with increasing frequency in the past two years (H. Seeliger, personal communication). The characteristics of the *Listeria* subgroups are listed in Table 4.

Intergeneric relationships of Listeria

The relationship of *Listeria* to the genera *Erysipelothrix*, *Gemella*, *Lactobacillus* and *Streptococcus* and to the taxon *Micro. thermosphactum* supports the results of previous numerical taxonomic studies (Davis *et al.*, 1969; Stuart & Pease, 1972; Jones, 1975*a*). However, the relationship is not close enough (Figs 1, 2 and 3) to justify including any of the *Listeria* subgroupings in any one of the above genera.

Microbacterium thermosphactum appears as a distinct group. This is in accord with the results of biochemical and DNA base ratio studies (Collins-Thompson *et al.*, 1972), and numerical taxonomic studies (Davis *et al.*, 1969; Stuart & Pease, 1972; Jones, 1975*a*), and with the recent proposal that strains of *Micro. thermosphactum* be placed in the new genus *Brochothrix* (Sneath & Jones, 1976).

The Lactobacillus strains clustered as four distinct subgroups, one of which (B₃) contained all the representatives of the homofermentative species *Lact. mali* isolated from cider (Carr & Davies, 1970). The other three subgroups corresponded closely to the subgenera proposed by Orla-Jensen (1919) and extended by Rogosa & Sharpe (1959).

The recovery of most of the Streptococcus strains in four main subgroups is in good agreement with the physiological divisions established by Sherman (1937) and extended by Sharpe, Fryer & Smith (1966).

With the exception of *Strep. durans* (G124) all the faecal streptococci formed one subgroup (B₆) with an internal structure corresponding to the 'enterococcus' and 'viridans' subdivisions of the faecal streptococci (Deibel, 1964; Jones, Sackin & Sneath, 1972). This subgroup showed the closest relationship of any of the *Streptococcus* subgroups to the genus *Listeria*, a result in agreement with those of Davis *et al.* (1969) and Stuart & Pease (1972). Of interest is the loose association of *Strep. uberis* ('viridans' division of Sherman, 1937) with subgroups B₅ and B₆. All but one of the *Strep. suis* strains (*Strep. suis* G141) clustered as subgroup C₇ and showed the closest association to a small subgroup containing *Strep. suis* (G141) and one strain each of serological groups M and O. The taxonomic position of *Strep. suis* is not clear. Elliott (1966) demonstrated that strains of *Strep. suis* contained the antigen of serological group D and suggested (Elliott, 1966) that *Strep. suis* was probably related to *Strep. bovis*. However, work in our laboratory has shown no close relationship between *Strep. suis* and *Strep. bovis* nor between *Strep. suis* and any of the other streptococci of serological group D (D. Jones, unpublished), and our present results agree with this opinion.

Buchanan & Gibbons (1974) include *Gemella haemolysans* and strains previously named *Neisseria haemolysans* in the genus *Gemella* in the family Streptococcaceae. Our results agree with this classification.

Erysipelothrix rhusiopathiae emerges as a distinct taxon showing no close relationship to the genus *Listeria*; indeed, it exhibits a much closer relationship to the subgroups formed by the genus *Gemella* and the streptococci. These findings are in agreement with the results of Davis *et al.* (1969) and Stuart & Pease (1972).

In the latest edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974) the genera *Erysipelothrix* and *Listeria* are treated as genera of uncertain taxonomic affiliation in Part 16 which also includes the family Lactobacillaceae. However, the family Streptococcaceae is listed in quite a different section and *Micro. thermosphactum* is included in the genus *Microbacterium* which is listed as a genus *incertae sedis* in the coryneform group.

Stuart & Welshimer (1974) have suggested the creation of a new family Listeriaceae to accommodate the monospecific genus *Listeria* (comprising *List. monocytogenes*) and the genus *Murraya* to contain the species *Murraya grayi* and *Murraya grayi* subsp. *murrayi*. If one accepts this, the result is three family groupings, Lactobacillaceae, Listeriaceae and Streptococcaceae, plus at least two other taxa, *Erysipelothrix* and *Micro. thermosphactum*, amongst the bacteria which cluster in area I of this study. A case can be made for the inclusion of *Erysipelothrix* in the family Streptococcaceae, although there would undoubtedly be opposition on the grounds of cell shape. However, the higher taxonomic relationship of

Micro. thermosphactum is more perplexing for it falls halfway between the listeriae and lactobacilli (see Fig. 3). It could be argued that *Micro. thermosphactum* is at present a solitary taxon which represents a number of as yet poorly studied bacteria. If this is the case, our results, and those of previous workers, indicate that we can expect even more blurring of the boundaries between the family Lactobacillaceae and the proposed family Listeriaceae (Stuart & Welshimer, 1974).

A good case can be made for including the taxa *Erysipelothrix*, *Gemella*, *Lactobacillus*, *Listeria*, *Streptococcus* and *Micro. thermosphactum* in one family: the Lactobacillaceae. Objections to this suggestion will probably be, firstly, that the suggested family grouping would embrace some taxa which have a rod-like cellular morphology and others which have a coccoid cell shape, and secondly, the grouping would contain some taxa which possess a fully functional cytochrome system and catalase and others which do not. Both cell shape and the presence of catalase have been regarded as important identification characters amongst groups of bacteria which have a very similar physiology. But how important taxonomically are these two characters? Many workers have commented on the effect of medium and age on the shape of bacteria; indeed, *List. monocytogenes* can be almost coccoid and is frequently mistaken for faecal streptococci on first isolation, while filaments are very common in old laboratory strains (Seeliger, 1961). Similarly, streptococci of serological group D may appear quite rod-like (Jones, 1959). Recent studies by London & Kline (1973) on the protein homologies of the malic enzymes and aldolases of rods and cocci in the lactic area indicated a close affinity between *Lact. casei* and *Strep. faecalis* and these authors concluded that shape is a relatively trivial character which should not be used to divide groups which are closely related on other criteria. In our opinion this applies more so when the shapes of individual bacteria are subject to quite marked variation.

For many years, the absence of catalase (and by implication, cytochromes) was considered a characteristic of the Streptococcaceae and the Lactobacillaceae. However, as pointed out by Smith (1954), the electron transport systems and associated enzymes of bacteria vary from species to species and with growth conditions. This has been shown to be true even of the lactic acid bacteria. Haem and non-haem catalases have been detected amongst this group (Felton, Evans & Niven, 1953; Wheeler, 1955; Whittenbury, 1960, 1964; Delwiche, 1961; Delwiche & Johnson, 1962) and, more recently, functional cytochromes have been detected in streptococci under certain growth conditions (Bryan-Jones & Whittenbury, 1969; Ritchie & Seeley, 1974). The catalase reaction of *Micro. thermosphactum* can be equivocal, although it contains a fully functional cytochrome system (Davidson & Hartree, 1968). The catalase reaction of *List. monocytogenes* depends on the composition of the basal medium (Jones, 1975*a*) and there is disagreement about whether this species contains cytochromes. Trivett & Meyer (1971) could not detect cytochromes in *List. monocytogenes* but Jones (unpublished results), using more sensitive methods, found low levels to be present.

These observations raise interesting evolutionary and taxonomic questions, as has already been pointed out by Bryan-Jones & Whittenbury (1969) with regard to the presence of cytochromes in streptococci, and in our opinion support the case for inclusion of all the taxa in Fig. 3 in one family, the Lactobacillaceae. Additionally this family might include the pediococci and leuconostocs not included in the present study. A study of the relationship of the hetero-fermentative cocci to the hetero-fermentative lactobacilli would be very interesting and it is a pity time did not allow their inclusion in this work.

CONCLUSIONS

1. The genus *Listeria* is a distinct taxon containing at least two species, *List. monocytogenes* and *List. grayi*. The relationships of certain non-haemolytic listeriae to the genus *Listeria* requires further study.
2. *Erysipelothrix* is quite distinct from *Listeria* and shows a close relationship to the streptococci.
3. Strains named *Microbacterium thermosphactum* form a distinct group worthy of genus rank. They are not related to the genus *Kurthia*.
4. The genus *Lactobacillus* contains four groupings corresponding to *Betabacterium*, *Streptobacterium*, *Thermobacterium* and *Lact. mali*.
5. The genus *Streptococcus* contains at least four distinct groups and all show a relatively close relationship to the genera *Gemella* and *Erysipelothrix*.
6. *Listeria* clusters with other lactic acid bacteria and in our opinion should be included in the family Lactobacillaceae together with the genera *Erysipelothrix*, *Gemella*, *Lactobacillus*, and *Streptococcus* and with the distinct taxon formed by strains of *Micro. thermosphactum*.

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