A Numerical Taxonomic Study of Coryneform and Related Bacteria

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SUMMARY

Two hundred and thirty-three strains of coryneform bacteria, including representatives of the genera *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Corynebacterium*, *Erysipelothrix*, *Jensenia*, *Kurthia*, *Listeria*, *Microbacterium*, *Mycobacterium*, *Nocardia* and *Propionibacterium* and other related bacteria, were studied using 173 morphological, physiological and biochemical tests. The bacteria were grown on a soil extract medium which allowed growth of all the strains, and all were incubated at 30 °C. The results were subjected to computer analysis. The majority of the strains grouped into eight main clusters representing: (A) *Lactobacillus*, *Listeria*, *Microbacterium thermosphactum* and *Streptococcus faecalis*; (B) *Erysipelothrix* and *Streptococcus pyogenes*; (C) animal corynebacteria and *Microbacterium flavum*; (D) *Cellulomonas* and related bacteria; (E) *Propionibacterium*; (F) *Arthrobacter*, *Brevibacterium*, *Kurthia* and *Mycobacterium rhodochrous*; (G) plant pathogenic corynebacteria; (H) *Nocardia*. Based on these clusters, several recommendations are made regarding the classification of the coryneform area. (i) The members of clusters A and B at present placed in the Corynebacteriaceae would seem better moved to the Lactobacillaceae. (ii) The genus *Corynebacterium* would best be retained for the type species *C. diphtheriae*, closely related animal corynebacteria and *Micro. flavum*. (iii) *Cellulomonas* and *Propionibacterium* are distinct taxa more closely related to *Corynebacterium* than to either *Arthrobacter* or *Lactobacillus*. (iv) Clusters F and G are evidently heterogeneous. In particular the positions of *Kurthia* and the plant pathogenic corynebacteria are unclear. *Arthrobacter* is a large loose taxon and it is premature to decide on its taxonomic rank. The genus *Brevibacterium* should be retained for *B. linens* and closely related strains. (v) The cellulolytic forms of *Nocardia* should be removed from the genus; they are however quite distinct from *Cellulomonas*.

INTRODUCTION

The term coryneform, originally used to describe the club-shaped or wedge-shaped cells of *Corynebacteria diphtheriae* and related animal parasites, is now widely used to describe Gram-positive, non-mycelial, non-sporing bacteria which exhibit a pleomorphic morphology (Jensen, 1952; Veldkamp, 1970).

The definitions of the genera commonly referred to as coryneform (*Corynebacterium*, *Microbacterium*, *Cellulomonas* and *Arthrobacter* (Jensen, 1952) are equivocal because they are based almost entirely on microscopic appearance and staining characteristics and these are affected by morphological changes during the growth cycle and also by culture conditions (Jensen, 1933; Cure & Keddle, 1972). Furthermore, some *Corynebacterium* species are morphologically similar to *Propionibacterium* and *Brevibacterium* while other members of the genus, together with the genera *Arthrobacter* and *Cellulomonas*, show a close

morphological resemblance to representatives of the genera *Mycobacterium* and *Nocardia* (Jensen, 1952; Skerman, 1967; Bowie, Grigor, Dunckley, Loutit & Loutit 1972).

The taxonomic situation is further complicated by the inclusion of the genera *Listeria* and *Erysipelothrix* in the family Corynebacteriaceae (*Bergey's Manual of Determinative Bacteriology*, 1957). These two genera do not exhibit coryneform morphology and, together with the species *Micro. thermosphactum*, are physiologically more closely related to the Lactobacillaceae (Davis Fomin, Wilson & Newton, 1969; Stuart & Pease, 1972; Collins-Thompson Sørhaug, Witter & Ordal, 1972; Trivett & Meyer, 1971; Wilkinson, 1973).

There are a number of reviews of the early literature (Clark, 1952; Jensen, 1934, 1952, 1966; Gibson, 1953; Veldkamp, 1970). In the past ten years there have been an increasing number of studies of the taxonomy of coryneform and possibly related bacteria by numerical taxonomic methods (Da Silva & Holt, 1965; Melville, 1965; Chatelain & Second, 1966; Harrington, 1966; Mullakhanbhai & Bhat, 1967; Splitstoesser, Wexler, White & Colwell, 1967; Seyfried, 1968; Davis *et al.* 1969; Davis & Newton, 1969; Masuo & Nakagawa, 1969; Skyring & Quadling, 1970; Bousfield, 1972; Vanderzant, Judkins, Nickelson & Fitshugh, 1972; Goodfellow, Fleming & Sackin, 1972) and by other techniques (Keddie, Leask & Grainger, 1966; Robinson, 1966*a, b, c*; Abe, Takayama & Kinoshita, 1967; Komagata, Yamada & Ogawa, 1969; Yamada & Komagata, 1968, 1970*a, b*, 1972*a, b*; Modarska, Modarski & Goodfellow, 1972). However, with a few exceptions (Stuart & Pease, 1972; Bousfield, 1972; Yamada & Komagata 1970*a, b*, 1972*a, b*) all have been concerned with only part of the coryneform area as circumscribed by Jensen (1952, 1966).

This study reports an attempt to establish the degree of similarity between the genera *Nocardia*, *Agromyces*, *Mycobacterium*, *Kurthia*, *Brevibacterium*, *Corynebacterium*, *Arthrobacter*, *Cellulomonas*, *Microbacterium*, *Listeria*, *Erysipelothrix*, *Propionibacterium*, *Lactobacillus* and *Streptococcus* by a numerical taxonomic study of a number of strains from these genera together with some unclassified bacteria. It was also hoped to clarify the validity of some or all of these genera, leading to a more convincing identification scheme for the coryneform bacteria.

METHODS

Strains. These are listed in Table 1. A few were fresh isolates, but the majority were received from other workers and from culture collections. Type strains were included wherever possible. Each strain was given a study reference number but the original strain designation is given in column 3 of Table 1. Several strains which showed colonial variation during preliminary culture were separated into discrete cultures which retained their characteristic morphology on further subculture over a period of years. Mucoid variants of NCIB8147 and G1257 were included in the study and are labelled NCIB8147 (m) and G1257 (m). Some strains of *Listeria* were obtained from different sources and it was later realized that they were in fact 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.

The strains were lyophilized and working cultures maintained by weekly subculture on the soil extract medium described below.

Basal medium. The selection of one basal medium which allowed growth of all the bacteria in the study posed a problem. At one extreme were *Arthrobacter* spp., some of which are inhibited by concentrations of peptone used in routine media and some of which also require 'terregens' factor for growth (Lockhead & Burton, 1956); at the other extreme were nutritionally demanding bacteria, e.g. streptococci, lactobacilli and some animal corynebacteria. Several media were tested. The best medium for this study was a modification of the medium

Table 1. *Strains used in the study*

Study reference number	Species	Strain designation	Source
C124	<i>Achromobacter liquefaciens</i>	ATCC15716	—
C265	<i>Agromyces ramosus</i>	ATCC25173	—
C80	<i>Arthrobacter atrocyaneus</i>	NCIB9220	—
C76	<i>Arth. aurescens</i>	NCIB8912	—
C79	<i>Arth. citreus</i>	NCIB8915	—
C122	<i>Arth. citreus</i>	M8909	J. G. Morris
C86	<i>Arth. crystallopoietes</i>	NCIB9499	—
C81	<i>Arth. duodecadis</i>	NCIB9222	—
C83	<i>Arth. flavescens</i>	NCIB9221	—
C71	<i>Arth. globiformis</i>	NCIB8602	—
C123	<i>Arth. globiformis</i>	M8911	J. G. Morris
C84	<i>Arth. nicotianae</i>	NCIB9458	—
C74	<i>Arth. oxydans</i>	NCIB9333	—
C75	<i>Arth. oxydans</i>	NCIB9334	—
C72	<i>Arth. pascens</i>	NCIB8910	—
C85	<i>Arth. ramosus</i>	NCIB9066	—
C73	<i>Arth. simplex</i>	NCIB8929	—
C120	<i>Arth. simplex</i>	M8913	J. G. Morris
C82	<i>Arth. terregens</i>	NCIB8909	—
C78	<i>Arth. tumescens</i>	NCIB8914	—
C77	<i>Arth. ureafaciens</i>	NCIB7811	—
C121	<i>Arth. ureafaciens</i>	M8916	J. G. Morris
C87	<i>Arth. variabilis</i>	NCIB9455	—
C130	<i>Arthrobacter</i> sp.	1	E. G. Mulder
C129	<i>Arthrobacter</i> sp.	4	E. G. Mulder
C133	<i>Arthrobacter</i> sp.	8	E. G. Mulder
C136	<i>Arthrobacter</i> sp.	11	E. G. Mulder
C131	<i>Arthrobacter</i> sp.	250	E. G. Mulder
C138	<i>Arthrobacter</i> sp.	251	E. G. Mulder
C126	<i>Arthrobacter</i> sp.	253	E. G. Mulder
C134	<i>Arthrobacter</i> sp.	326	E. G. Mulder
C135	<i>Arthrobacter</i> sp.	328	E. G. Mulder
C137	<i>Arthrobacter</i> sp.	329	E. G. Mulder
C128	<i>Arthrobacter</i> sp.	365	E. G. Mulder
C127	<i>Arthrobacter</i> sp.	367	E. G. Mulder
C132	<i>Arthrobacter</i> sp.	368	E. G. Mulder
C140	<i>Arthrobacter</i> sp.	C140	M. Goodfellow
C141	<i>Arthrobacter</i> sp.	C141	M. Goodfellow
C143	<i>Arthrobacter</i> sp.	C143	M. Goodfellow
C159	<i>Arthrobacter</i> sp.	G93	R. M. Keddie
C160	<i>Arthrobacter</i> sp.	G109	R. M. Keddie
C161	<i>Arthrobacter</i> sp.	G143	R. M. Keddie
C162	<i>Arthrobacter</i> sp.	G154	R. M. Keddie
C163	<i>Arthrobacter</i> sp.	G162	R. M. Keddie
C164	<i>Arthrobacter</i> sp.	G187	R. M. Keddie
C165	<i>Arthrobacter</i> sp.	H72	R. M. Keddie
C166	<i>Arthrobacter</i> sp.	H123	R. M. Keddie
C167	<i>Arthrobacter</i> sp.	J30	R. M. Keddie
C168	<i>Arthrobacter</i> sp.	J39	R. M. Keddie
C169	<i>Arthrobacter</i> sp.	J41	R. M. Keddie
C170	<i>Arthrobacter</i> sp.	J52	R. M. Keddie
C171	<i>Arthrobacter</i> sp.	J62	R. M. Keddie
C172	<i>Arthrobacter</i> sp.	J93	R. M. Keddie
C236	<i>Arthrobacter</i> sp.	TG1	J. G. Morris
C246	<i>Arthrobacter</i> sp.	A137/68	L. R. Hill
C250	<i>Arthrobacter</i> sp.	Brzin	B. Brzin
C207	<i>Bacterium eurydice</i>	C207	L. Bailey
C208	<i>Ba. eurydice</i>	C208	L. Bailey

Table 1 (cont.)

Study reference number	Species	Strain designation	Source
C11	<i>Ba. eurydice</i>	C11	L. Bailey
C26	<i>Brevibacterium acetylicum</i>	ATCC953	—
C93	<i>Br. ammoniogenes</i>	NCIB8143	—
C94	<i>Br. divaricatum</i>	NCIB9379	—
C25	<i>Br. imperiale</i>	ATCC8365	—
C24	<i>Br. incertum</i>	ATCC8363	—
C23	<i>Br. leucinophagum</i>	ATCC13809	—
C21	<i>Br. linens</i>	ATCC9174	—
C92	<i>Br. linens</i>	NCIB8546	—
C22	<i>Br. stationis</i>	ATCC14403	—
C95	<i>Cellulomonas biazotea</i>	NCIB8077	—
C100	<i>Cel. bibula</i>	NCIB8142	—
C96	<i>Cel. cellulasea</i>	NCIB8078	—
C97	<i>Cel. fimi</i>	NCIB8980	—
C99	<i>Cel. flavigena</i>	NCIB8073	—
C98	<i>Cel. gelida</i>	NCIB8076	—
C101	<i>Cel. rossica</i>	NCIB8074	—
C102	<i>Cel. subalbus</i>	NCIB8075	—
C27	<i>Cel. uda</i>	ATCC491	—
C67	<i>Corynebacterium aquaticum</i>	NCIB9460	—
C35	<i>Cor. betae</i>	NCPPB363	—
C13	<i>Cor. bovis</i>	ATCC7715	—
C41	<i>Cor. diphtheriae</i>	NCTC3984	—
C223	<i>Cor. diphtheriae</i>	NCTC3985	—
C224	<i>Cor. diphtheriae</i>	NCTC3989	—
C225	<i>Cor. diphtheriae</i>	Bergan 8	P. H. A. Sneath
C48	<i>Cor. equi</i>	NCTC1621	—
C17	<i>Cor. fascians</i>	ATCC12974	—
C36	<i>Cor. fascians</i>	NCPPB1488	—
C37	<i>Cor. flaccumfasciens</i>	NCPPB559	—
C45	<i>Cor. haemolyticum</i>	NCTC8452	—
C10	<i>Cor. hofmanii</i>	S45	C. S. Cummins
C18	<i>Cor. ilicis</i>	ATCC14264	—
C38	<i>Cor. insidiosum</i>	NCPPB83	—
C68	<i>Cor. manihot</i>	NCIB9097	—
C69	<i>Cor. mediolanum</i>	NCIB7206	—
C34	<i>Cor. michiganense</i>	NCPPB1468	—
C65	<i>Cor. minutissimum</i>	NCTC10284	—
C66	<i>Cor. minutissimum</i>	NCTC10288	—
C47	<i>Cor. murium</i>	NCTC949	—
C15	<i>Cor. nephridii</i>	ATCC11425	—
C42	<i>Cor. ovis</i>	NCTC3450	—
C14	<i>Cor. paurometabolum</i>	ATCC8368	—
C40	<i>Cor. poinsettiae</i>	NCPPB844	—
C44	<i>Cor. pyogenes</i>	NCTC5224	—
C249	<i>Cor. pyogenes</i>	C249	N. S. Mair
C39	<i>Cor. rathayi</i>	NCPPB797	—
C46	<i>Cor. renale</i>	NCTC7448	—
C70	<i>Cor. rubrum</i>	NCIB9433	—
C16	<i>Cor. vesiculare</i>	ATCC11426	—
C64	<i>Cor. viscosum</i>	NCTC2416	—
C43	<i>Cor. xerosis</i>	NCTC9755	—
C63	<i>Corynebacterium</i> sp.	NCTC7510	—
C239	<i>Corynebacterium</i> sp.	U1135	H. R. Carne
C206	<i>Corynebacterium</i> sp.	C206	C. Ratledge
C180	<i>Corynebacterium</i> sp.	3-8	H. E. Schefferle
C176	<i>Corynebacterium</i> sp.	6-10	H. E. Schefferle
C181	<i>Corynebacterium</i> sp.	7-11	H. E. Schefferle

Table 1 (*cont.*)

Study reference number	Species	Strain designation	Source
C177	<i>Corynebacterium</i> sp.	8-7	H. E. Schefferle
C179	<i>Corynebacterium</i> sp.	10-21	H. E. Schefferle
C178	<i>Corynebacterium</i> sp.	11-10	H. E. Schefferle
C191	<i>Corynebacterium</i> sp.	3A 4302	C. Shaw
C189	<i>Corynebacterium</i> sp.	3A 4429	C. Shaw
C190	<i>Corynebacterium</i> sp.	3A 4555	C. Shaw
C187	<i>Corynebacterium</i> sp.	3A 4669	C. Shaw
C188	<i>Corynebacterium</i> sp.	3A 4675	C. Shaw
C186	<i>Corynebacterium</i> sp.	3A 4715	C. Shaw
C185	<i>Corynebacterium</i> sp.	3A 4794	C. Shaw
C182	<i>Corynebacterium</i> sp.	4C 1528	C. Shaw
C184	<i>Corynebacterium</i> sp.	4D 9312	C. Shaw
C183	<i>Corynebacterium</i> sp.	3W 8957	C. Shaw
C243	<i>Corynebacterium</i> sp.	NCIB8179	—
C245	<i>Corynebacterium</i> sp.	NCIB8181	—
C244	<i>Corynebacterium</i> sp.	NCIB8180	—
C118	<i>Diphtheroid</i> Q	NCTC7420	—
C50	<i>Erysipelothrix rhusiopathiae</i>	NCTC8163	—
C220	<i>E. rhusiopathiae</i>	Sneath 19	P. H. A. Sneath
C221	<i>E. rhusiopathiae</i>	Sneath 45	P. H. A. Sneath
C219	<i>E. rhusiopathiae</i>	Sneath 49	P. H. A. Sneath
C218	<i>E. rhusiopathiae</i>	Sneath 53	P. H. A. Sneath
C217	<i>E. rhusiopathiae</i>	Sneath 115	P. H. A. Sneath
C107m	<i>Jensenia canicruria</i>	NCIB8147m	—
C107c	<i>J. canicruria</i>	NCIB8147	—
C51	<i>Kurthia zopfii</i>	NCTC404	—
C91	<i>K. zopfii</i>	NCIB8603	—
C5	<i>K. zopfii</i>	K2	R. M. Keddie
C6	<i>K. zopfii</i>	s8	R. M. Keddie
C7	<i>K. zopfii</i>	s9	R. M. Keddie
C205	<i>Kurthia</i> sp.	8	G. A. Gardner
C125	<i>Kurthia</i> sp.	F64/100	E. M. Barnes
C232	<i>Lactobacillus casei</i> var <i>rhamnosus</i>	NCDO243	—
C233	<i>Lact. plantarum</i>	NCDO1752	—
C210	Atypical <i>Lactobacillus</i>	LTRS MT1	M. E. Sharpe
C209	Atypical <i>Lactobacillus</i>	LTRS MT7	M. E. Sharpe
C211	Atypical <i>Lactobacillus</i>	LTRS MT36	M. E. Sharpe
C212	<i>Listeria denitrificans</i>	L26	Pasteur Institute, Paris
C213	<i>List. grayi</i>	Seeliger L332/64	H. L. Seeliger
C231	<i>List. monocytogenes</i>	NCTC4883	—
C226	<i>List. monocytogenes</i>	NCTC4885	—
C203	<i>List. monocytogenes</i>	NCTC4885 (Mair)	N. S. Mair
C227	<i>List. monocytogenes</i>	NCTC5105	—
C202	<i>List. monocytogenes</i>	NCTC5105 (Mair)	N. S. Mair
C228	<i>List. monocytogenes</i>	NCTC5214	—
C229	<i>List. monocytogenes</i>	NCTC5343	—
C200	<i>List. monocytogenes</i>	NCTC5348	—
C52	<i>List. monocytogenes</i>	NCTC7973a	—
C230	<i>List. monocytogenes</i>	NCTC7973	—
C201	<i>List. monocytogenes</i>	NCTC10357	—
C198	<i>List. monocytogenes</i>	Mair C198	N. S. Mair
C199	<i>List. monocytogenes</i>	Mair 26431	N. S. Mair
C197	<i>List. monocytogenes</i>	Mair 28251	N. S. Mair
C216	<i>List. monocytogenes</i>	Mair C216	N. S. Mair
C196	<i>List. monocytogenes</i>	Mair C196	N. S. Mair
C235	<i>List. monocytogenes</i>	MRC C235	—
C242	<i>List. monocytogenes</i>	Brzin	B. Brzin
C248	<i>List. monocytogenes</i>	Mair C248	N. S. Mair

Table 1 (cont.)

Study reference number	Species	Strain designation	Source
C213	<i>List. monocytogenes</i>	Potel 188/7	H. L. Seeliger
C90	<i>Microbacterium flavum</i>	NCIB8707	—
C88	<i>Micro. lacticum</i>	NCIB8540	—
C89	<i>Micro. lacticum</i>	NCIB8541	—
C20	<i>Micro. thermosphaetum</i>	ATCC11509	—
C2	<i>Micro. thermosphaetum</i>	CR 12A	A. G. Kitchell
C3	<i>Micro. thermosphaetum</i>	30	A. G. Kitchell
C4	<i>Micro. thermosphaetum</i>	30A	A. G. Kitchell
C62	<i>Micrococcus luteus</i>	NCTC8512	—
C29	<i>Mycobacterium fortuitum</i>	ATCC6841	—
C57	<i>Myco. phlei</i>	NCTC8151	—
C58	<i>Myco. smegmatis</i>	NCTC8159	—
C173	<i>Myco. rhodochrous</i>	NCTC8139	—
C174	<i>Myco. rhodochrous</i>	NCTC10210	—
C12	<i>Myco. rhodochrous</i>	ATCC13808	—
C153	<i>Myco. rhodochrous</i>	G21	R. E. Gordon
C157	<i>Myco. rhodochrous</i>	G417	R. E. Gordon
C156	<i>Myco. rhodochrous</i>	G463	R. E. Gordon
C155	<i>Myco. rhodochrous</i>	G768	R. E. Gordon
C144	<i>Myco. rhodochrous</i>	G817	R. E. Gordon
C148	<i>Myco. rhodochrous</i>	G1240	R. E. Gordon
C145	<i>Myco. rhodochrous</i>	G1256	R. E. Gordon
C147m	<i>Myco. rhodochrous</i>	G1257M	R. E. Gordon
C147c	<i>Myco. rhodochrous</i>	G1257	R. E. Gordon
C146	<i>Myco. rhodochrous</i>	G1293S	R. E. Gordon
C151	<i>Myco. rhodochrous</i>	G1293R	R. E. Gordon
C154	<i>Myco. rhodochrous</i>	G3048	R. E. Gordon
C158	<i>Myco. rhodochrous</i>	G7698	R. E. Gordon
C152	<i>Myco. rhodochrous</i>	ATCC12975	R. E. Gordon
C149	<i>Nocardia brasiliensis</i>	G744	R. E. Gordon
C111	<i>N. calcaraea</i>	NCIB8863	—
C55	<i>N. caviae</i>	NCTC1934	—
C110	<i>N. cellulans</i>	NCIB8868	—
C109	<i>N. coeliaca</i>	NCIB8939	—
C53	<i>N. farcinica</i>	NCTC4524	—
C112	<i>N. hydrocarboxydans</i>	NCIB9436	—
C54	<i>N. madurae</i>	NCTC5654	—
C108	<i>N. opaca</i>	NCIB9409	—
C150	<i>N. pelletieri</i>	G513	R. E. Gordon
C113	<i>N. petroleophila</i>	NCIB9438	—
C114	<i>N. rugosa</i>	NCIB8926	—
C56	<i>N. salivae</i>	NCTC10207	—
C115	<i>N. saturnea</i>	NCIB9437	—
C194	<i>N. turbata</i>	C194 (strain A)	D. M. Webley
C193	<i>N. turbata</i>	C193 (strain B)	D. M. Webley
C192	<i>N. turbata</i>	C192 (strain C)	D. M. Webley
C28	<i>N. vaccinii</i>	ATCC11092	—
C142	<i>Nocardia</i> sp.	C142	M. Goodfellow
C104	<i>Propionibacterium freundenreichii</i>	NCIB5959	—
C116	<i>P. jensenii</i>	NCIB5960	—
C106	<i>P. pentosaceum</i>	NCIB8070	—
C105	<i>P. petersonii</i>	NCIB5962	—
C103	<i>P. shermanii</i>	NCIB8099	—
C117	<i>Streptococcus faecalis</i>	NCIB6782	—
C32	<i>Strep. faecalis</i>	T91	R. H. Deibel
C33	<i>Strep. faecalis</i>	P318	R. H. Deibel
C9	<i>Strep. pyogenes</i>	S6	C. S. Cummins
C61	<i>Streptococcus</i> sp. (serological group Q)	NCTC6198	—

of Topping (1937). This contained: neopeptone (Difco), 2.5 g; yeast extract (Difco), 2.5 g; glucose, 2.5 g; Tween 80 (Koch-Light), 1 g; agar (Davies), 15 g; soil extract, 250 ml; and distilled water to a final volume of 1 l, at pH 6.8. The soil extract was prepared by suspending 500 g air-dried garden soil in 1 l tap water and autoclaving at 120 °C for 15 min. After sedimentation of the larger particles, the hot supernatant was decanted and clarified by filtration through paper pulp in a Buchner funnel. This procedure produced a clear soil extract which was used in the medium described above. The medium minus agar was also used as a broth. These media, subsequently referred to as SEM agar and SEM broth, were used as the basal medium in most of the tests. Different media minus soil extract were used with certain strains to check the effect of soil extract on the tests, but in no case were the tests greatly affected. All tests were incubated at 30 °C unless otherwise stated.

Colonial and cell morphology. The bacteria were grown on SEM agar and examined under a low-power microscope for colonial morphology after 1, 2, 4 and 7 days at $\times 100$ and $\times 400$ magnifications. Gram stains were performed at the same times and examined at $\times 1000$ magnification. Spore stains were done on 7 day cultures. Acid fastness was investigated using 5 day cultures grown on SEM agar supplemented with glycerol (7 %, w/v).

Pigment production was noted in colonies examined for colonial morphology. After 7 days the cultures were removed from the incubator and left in light at room temperature for 14 days.

Growth characteristics in liquid medium were examined in SEM broth at 1, 2, 4 and 7 days.

Ability to grow on and/or haemolyse blood was tested by culturing the bacteria on blood agar base (Oxoid) plus 7 % (v/v) sterile horse blood. Petri dishes were examined daily for 5 days for growth and type of haemolysis.

Henry technique (iridescence). Twenty-four h Petri dish cultures of bacteria on tryptose agar (Difco) were examined microscopically with obliquely transmitted white light following the technique of Henry (1933) as described by Wood (1969). *Listeria* spp. appear as small, low convex colonies with a bright blue-green iridescence.

Growth at different temperatures. Bacteria were incubated in tubes of SEM broth at 4, 10, 25, 30, 37, 45 and 50 °C in water baths maintained at the given temperature ± 1 °C. Turbidity was scored as slight, moderate or dense for up to 7 days, or up to 21 days for growth at 4 and 10 °C.

Survival at 60 °C for 30 min. Tubes of SEM broth (preheated to 60 °C) were inoculated with 2 loopfuls (4.0 mm diam) of 18 h cultures, held at 60 °C for 30 min, cooled immediately, incubated at 30 °C and examined for growth at intervals for 7 days.

Motility. SEM medium (agar reduced to a concentration of 0.25 %) was dispensed into wide-necked 1 oz Universal bottles each containing a Craigie tube. The inner tube was inoculated with the bacterium under study, then incubated at room temperature. The medium surrounding the tube was examined for surface growth and microcolonies in the outer region at frequent intervals for up to 6 weeks. Motile cultures were stained for flagella by the method of Rhodes (1958).

Catalase, benzidine, oxidase. Bacterial growth on SEM agar Petri dishes was used for these tests. Any strain giving a negative result after 24 h growth was retested at 48 h and in some cases at 3 to 5 days. H_2O_2 (20 vol.) was used to test for catalase; the benzidine test was carried out by the method of Deibel & Evans (1960); oxidase was tested for by the method of Klinge (1960), strong oxidase-positive reactions being recorded after 10 s and weak ones after 30 s to 1 min.

Ammonia from peptone. Small portions of broth were removed from SEM broth after

7 and 21 days, placed on a white tile, and 3 drops of Nessler's reagent (BDH) added. A yellow or orange coloration was scored as positive.

Sensitivity to antibiotics. SEM agar and blood agar base (Oxoid) were used. The Petri dishes 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 Ltd, Liverpool) were then dropped on to the dishes (4 per dish). The test dishes were observed after 24 h and 48 h incubation and again at 3 to 5 days in the case of the slower-growing bacteria. Zones of total and partial inhibition and also the growth of mutant colonies were recorded.

Cellulose hydrolysis. Cellulolytic activity was investigated by inoculating strains into tubes of SEM broth containing strips of Whatman No. 1 filter paper, half the length of which was above the level of the liquid. The tubes were observed at frequent intervals for 9 weeks. A positive result was recorded if the filter paper disintegrated completely at the liquid/air interface.

Gelatin hydrolysis. SEM broth plus 15 % (w/v) gelatin (Oxoid) dispensed in 10 ml quantities was inoculated by straight wire. After incubation at 30 °C the tests were observed at frequent intervals for 6 weeks. Before reading, the tubes were refrigerated at 4 °C for 1 h to solidify the unaffected gelatin.

Chitin hydrolysis. Chitin (BDH) was ground in a ball mill to produce a fine powder, then incorporated into SEM agar to give a concentration of 0.5 % (w/v). After sterilization at 120 °C for 15 min, the chitin was evenly dispersed through the molten agar before dispensing into Petri dishes. Cultures were streaked in one line (two strains/dish) of growth. The dishes were incubated and observed for areas of clearing at intervals for nine weeks.

Casein hydrolysis. An equal volume of 10 % (w/v) skim milk (Oxoid) in distilled water was added to SEM agar (2 × normal agar concentration). Some bacteria were also tested on SEM agar minus glucose and on blood agar base plus skim milk. The Petri dishes were prepared, inoculated and read as for chitin hydrolysis for 21 days. There was no difference in the results with those bacteria tested on the three media.

Egg yolk reaction. Sterilized SEM medium was allowed to cool to approximately 50 °C. Egg yolk emulsion (Oxoid) previously heated to 50 °C was then added to give a final concentration of 10 % (v/v). The medium was mixed, dispensed into Petri dishes and inoculated as described for chitin hydrolysis. Dishes were examined for the appearance of opalescence around the colonies for 21 days.

Deoxyribonuclease. Bacteria were grown on SEM agar plus 2 % (w/v) deoxyribonucleic acid and 0.08 % (w/v) CaCl₂, and most were also tested on the medium of Di Salvo (1958). The Petri dishes were inoculated as described under chitin hydrolysis. After 5 days (10 days for slow-growing bacteria) the dishes were flooded with 1 N-HCl and inspected for areas of clearing.

Urease. The medium consisted of 50 ml 20 % (v/v) urea (sterilized by membrane filtration), added to 500 ml of sterilized molten SEM agar minus yeast extract and glucose and with the peptone concentration reduced to 0.01 % but with 0.012 g of phenol red as indicator; this was dispensed in 10 ml quantities into test tubes and allowed to set as slopes. After inoculation the tubes were inspected daily for 21 days for development of red colour.

Tween hydrolysis. Tween 20, 40, 60 and 80 (Koch-Light), at final concentrations of 1 % (v/v), were added to SEM agar minus Tween 80, plus 0.01 % (w/v) CaCl₂. After sterilization at 120 °C for 15 min the medium was dispensed into Petri dishes and inoculated as described for chitin hydrolysis. Zones of precipitation of the calcium salt and zones of clearing were recorded. In some cases both phenomena occurred together, in others separately. The mechanism of clearing on these media is unknown.

Aesculin. The medium and method of Sneath (1966) were used, except that the medium was made up in 75 % distilled water and 25 % soil extract. Blackening and loss of purple fluorescence were recorded; the former sometimes occurred without loss of purple fluorescence.

Arginine. (i) SEM broth plus 0.3 % (w/v) arginine distributed in 5 ml amounts in test tubes was used. After inoculation, drops were aseptically removed for periods up to 21 days and tested with Nessler's reagent. (ii) Thornley's arginine medium (Thornley, 1960) modified by the inclusion of 25 % soil extract was used.

Xanthine. SEM agar plus 0.5 % (w/v) xanthine autoclaved at 120 °C for 15 min was dispensed into Petri dishes. The dishes were inoculated as described for chitin and examined for areas of clearing for 21 days.

Tyrosine. SEM agar plus 0.5 % (w/v) L-tyrosine autoclaved at 120 °C for 15 min was dispensed into Petri dishes, care being taken to ensure even distribution of the tyrosine. The dishes were inoculated as described for chitin hydrolysis and inspected for clearing due to decomposition of the tyrosine or production of brown pigment, or both, at intervals up to 21 days. Some of the bacteria were also tested on blood agar base medium (Oxoid) plus tyrosine. The results were identical on both media.

Nitrate reduction. Bacteria were inoculated into SEM broth plus 0.1 % (w/v) KNO₃, dispensed in 10 ml quantities in tubes containing an inverted Durham tube. The tubes were examined at frequent intervals for 21 days for the presence of gas in the Durham tube. At the same time samples were removed and tested for the presence of nitrite and hydroxylamine by the methods described by Wilson & Miles (1964). Negative tests were checked at 21 days by the addition of zinc dust to reduce residual nitrate to nitrite.

Hippurate hydrolysis. SEM broth samples containing 1 % (w/v) sodium hippurate in 10 ml quantities in 1 oz bottles were inoculated and incubated for 21 days. Each broth was then centrifuged, and two 1 ml samples were removed; to one was added 0.5 ml of 12 % (w/v) ferric chloride and to the other 1 ml 50 % (v/v) sulphuric acid (Davis, 1955). Both test methods were used because weak positive reactions are frequently only detected by sulphuric acid.

Methyl red and Voges Proskauer (VP) tests. Two media were used: SEM broth containing 0.5 % (w/v) glucose; Hartley's digest broth (Oxoid) plus 0.5 % (w/v) K₂HPO₄ and 0.5 % (w/v) glucose. Both media were dispensed in 10 ml quantities. After inoculation the broths were tested at 2, 4 and 7 days as described by Cowan & Steel (1965), Barritt's method being used for the VP reaction.

Production of acid from carbohydrates. Difficulty was experienced with yeast extract (Difco and Oxoid). Acid was produced by a number of lactic acid bacteria, notably the streptococci and *Listeria* spp., from the basal medium minus any added carbohydrate. At first the soil extract was suspect, but then tests indicated it was probably due to the trehalose content of the yeast extract and it was overcome by lowering the concentration of this constituent to 0.1 % (w/v) and always including a sugarless control.

Two basal media were used. (i) Peptone (Difco), 15 g; yeast extract (Difco), 1 g; Tween 80, 1 g; water, 1 l. (ii) SEM broth minus glucose, and with the yeast extract concentration reduced to 0.1 %. In both cases the indicator was 3 ml of 1 % aqueous brom-cresol purple/l media. The appropriate carbohydrates were added as filter-sterilized solutions to the sterilized basal media to give a final concentration of 1 %.

The tests were also carried out in two ways: in test tubes, and in divided dishes (Sneath & Stevens, 1967). When tests were performed in divided dishes, 0.5 % agar (standard agar Davies) was added. The tubes were incubated at 30 °C, but the divided dishes were incubated

at 23 °C because at 30 °C some strains formed large quantities of mucoid material which 'crept' over the divisions in the dishes. All tests were read daily for 21 days.

Oxidative/fermentative utilization of glucose. The method of Hugh & Leifson (1953) was used, except that the two basal media were those described above. Additionally, one set of tubes was incubated in an anaerobic jar under an atmosphere of hydrogen rather than with the Vaseline seal.

Starch hydrolysis. A 10% (w/v) aqueous starch solution was dissolved by heating and added to SEM agar minus glucose to give a final concentration of 0.2% (w/v). Petri dishes were inoculated as described for chitin hydrolysis. After 7 to 10 days of incubation the dishes were flooded with iodine solution and examined for clearing around and under the growth.

Phosphatase. A 1% (w/v) aqueous solution of phenolphthalein diphosphate (Na salt; BDH) sterilized by filtration was added to molten SEM agar to give a final concentration of 0.001% (w/v). The medium was dispensed in Petri dishes and inoculated with bacteria so that discrete colonies were obtained. After 7 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 coloration around the colonies due to the free phenolphthalein reacting with the ammonia vapour.

Sulphatase. The medium and method used was as described for 'phosphatase', phenolphthalein disulphate (K salt; Koch-Light) being substituted for the diphosphate.

Potassium thiocyanate. SEM agar containing potassium thiocyanate (2.5, 3.75 and 4.5%) was dispensed in divided dishes and inoculated as described by Stevens (1969). Growth was compared with that on SEM agar minus potassium thiocyanate for 7 days.

Potassium tellurite. An aqueous solution of potassium tellurite (10%, w/v), sterilized by autoclaving at 120 °C for 15 min, was added to cooled SEM agar to give final concentrations of 0.01, 0.025 and 0.05%. The medium was dispensed and inoculated as for potassium thiocyanate. Growth and degree of blackening were compared with that on SEM agar minus potassium tellurite for 7 days.

Thallous acetate. SEM agar containing thallous acetate (0.01, 0.025 and 0.05%) was dispensed, inoculated and growth recorded as for potassium thiocyanate.

Sodium azide. SEM agar containing sodium azide (0.01, 0.02 and 0.05%) was dispensed, inoculated and growth recorded as for potassium thiocyanate.

Tetrazolium. An aqueous solution of 2,3,5-triphenyltetrazolium chloride (10%, w/v) was prepared by steaming for 30 min and added to previously sterilized molten SEM agar to give final concentrations of 0.01 and 0.1%. The medium was dispensed and inoculated as described for potassium thiocyanate. Growth and reduction of tetrazolium were compared with that on SEM agar for 7 days.

Litmus milk. Litmus milk (Oxoid) was reconstituted as directed by the manufacturers except that 25% (v/v) soil extract was used in the distilled water. Cultures were examined daily for reduction before clotting, acid production, alkaline production, clotting and digestion for 21 days.

Utilization of organic acids and glucose as carbon sources. Two methods were used. (i) SEM broth minus glucose, but plus the substrate under test to a concentration of 1 and 10% (w/v), was dispensed into matched test tubes. Growth in SEM broth and SEM broth plus the substrate were compared at 1, 2, 4, 7 and 14 days by absorbance readings in a Bausch & Lomb spectrophotometer at 550 nm. Positive results were scored only when the growth in the broth plus substrate was at least twice that in the basal broth, as many of the strains showed some growth in SEM broth alone. (ii) Medium and method of Gordon & Mihm

(1957); incubation and readings as described above. In some cases the medium was supplemented with soil extract (25 %, v/v).

Indole. SEM broth with tryptone substituted for peptone was used. The broths were incubated 14 days and tested by method 2 of Cowan & Steel (1965).

Hydrogen sulphide production. Slopes of SEM medium supplemented with 0.05 % (w/v) cysteine hydrochloride (BDH) were inoculated with 24 h cultures of the test bacteria. Strips of lead acetate paper inserted between the cap and the tube were observed for blackening. The tubes were incubated for 24 days but most positive reactions occurred between 2 and 5 days.

Ability to use NH_4^+ and NO_3^- as sole nitrogen sources. The method of Owens & Keddie (1969) was used.

Susceptibility to lysozyme. Bacteria were grown on SEM plates for 48 h at 30 °C. The growth was removed, resuspended in 1.5 ml distilled water, and divided into three parts in 10 × 1.2 cm test tubes. To one set was added 0.5 ml distilled water, to the second 0.5 ml lysozyme solution (100 µg lysozyme/ml) and to the third 0.5 ml lysozyme plus EDTA solution (100 µg lysozyme and 0.5 mg EDTA/ml) (Metcalf & Deibel, 1969). All the tubes were incubated at 35 °C for 90 min, and examined for lysis by clearing and for the appearance of a glutinous mass. An equal volume of NaCl (3 %, w/v) was then added to each tube, which was immediately inspected for lysis as described above. Controls were included in all tests and all tests were repeated at least twice.

Coding of characters. Except for the ability to grow on 7 % (v/v) blood agar, all the results were scored from those media containing soil extract. The Gram reaction was not scored because all strains showed some degree of Gram-positive staining although some strains appeared more Gram-negative. However, this trait was not reproducible, and the mode of regulation of citrate synthase (Jones & Weitzman, 1971) showed some but not all of these Gram-variable strains to have citrate synthases characteristic of Gram-negative bacteria. These strains are discussed below.

Those characters which existed in one or two mutually exclusive states were scored 1 (plus) or 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 computed and clustered by both single linkage (Sneath, 1957) and unweighted pair-group average linkage (Sokal & Michener, 1958). The I GROUPS (integer groups) program printed character-value statistics on specified groups of bacteria. This program, devised by M. J. Sackin of this Unit, aided the selection of tests important in the characterization of taxonomic groups.

As the maximum number of strains that could be computed at one time was 180, two runs had to be made. This was done by putting all the facultative anaerobic bacteria plus a selection of other strains into one run, and all the aerobic bacteria plus a selection of facultative anaerobic strains into the second run.

All calculations were carried out on the ICL 4130 computer at Leicester University.

RESULTS

Clustering

As noted in Methods, it was necessary to split the strains into two groups for computation. Some of the strains were included in both computer runs but the relative numbers from different genera were different in each.

Deviations from the published descriptions were found in a number of the strains studied. Strain LTRSM7 (atypical lactobacilli, Group 1 of Thornley & Sharpe, 1959) was strongly catalase-positive and weakly benzidine-positive. *Achromobacter liquefaciens* (ATCC15716) was found to be Gram-positive whereas *Brevibacterium leucinophagum* (ATCC13809) was Gram-negative (Jones & Weitzman, 1974). As noted by Jones, Watkins & Meyer (1970), *Agromyces ramosus* differed in several ways from the published description.

Most of the 233 strains grouped in 8 clusters (A, B, C, D, E, F, G, H) at the 75 % similarity level (% S). The quantitative taxonomic relationships within and between the clusters are illustrated in the sorted similarity matrices (Figs. 1 and 2). Dendrograms derived from these matrices are shown in Figs. 3 and 4. Six strains remained outside the main groups formed at 75 % S in both computer runs. These were strains received as *Ach. liquefaciens* (ATCC15716), *Brevibacterium acetylicum* (ATCC953), *Br. leucinophagum* (ATCC13809), *Corynebacterium nephridii* (ATCC11425), *Cor. insidiosum* (NCPB83) and *Cellulomonas rossica* (NCIB8074).

There was good accord between the clusters obtained in the two computations although some strains moved position. In interpreting the results of the clustering the main information was taken from the computer run containing the greater number of strains in a particular cluster, but information pertinent to the validity and stability of that cluster was also taken from the grouping of the same strains (if they were included) in the other computation. Cluster B (*Erysipelothrix* and some streptococci) appeared in only one computer run (Figs. 1 and 3). Cluster H (*Nocardia*, *Mycobacterium*) also appeared in one computation only (Figs. 2 and 4) but the situation is different because three of the 16 strains were included in the other computation where they joined Cluster F (Figs. 1 and 3).

Cluster A (*Lactobacillus*, *Listeria*, *Microbacterium*, *Streptococcus*). Above 80 % S this cluster contained four subgroups (Figs. 1 and 3). A1, with an internal similarity of 90 % S, contained five strains received as *Micro. thermosphactum* and one strain received as an atypical *Lactobacillus* sp. (LTRS MT7). A2 contained *Lact. casei*, *Lact. plantarum* and strain LTRS MT1, also received as an atypical *Lactobacillus* sp. A3 contained 20 strains received as *List. monocytogenes*, one strain received as *List. grayi*, one received as *Erysipelothrix rhusiopathiae* (this was a misnamed strain of *List. monocytogenes* and serological tests confirmed its position), and an atypical *Lactobacillus* sp., LTRS MT36. A4 contained three strains received as *Strep. faecalis*. The position was only slightly different in the other computer run (Figs. 2 and 4) where only eight of these strains were included.

Cluster B (*Erysipelothrix*, *Streptococcus*). This was a small cluster containing only seven strains (Figs. 1 and 3). Four of the strains received as *E. rhusiopathiae* showed a similarity greater than 95 %. These joined *E. rhusiopathiae* (NCTC8163) and a *Streptococcus* strain of serological group G at 83 % S, and all joined one strain of *Strep. pyogenes* at 80 % S.

Cluster C (animal corynebacteria, *Micro. flavum*, some arthrobacters). At about 77 % S this cluster contained two subgroups (Figs. 1 and 3). C1 contained 13 strains: 12 animal *Corynebacterium* spp. and *Micro. flavum*. C2 contained a heterogeneous collection of ten strains received as animal *Corynebacterium* spp., '*Bacterium eurydice*', *Brevibacterium incertum*, *Arthrobacter terregens* and *Agromyces ramosus*. In the other computation (Figs. 2 and 4), subgroup C2 did not exist because only *Arth. terregens* and *Agr. ramosus* were

included, but the relationship between the strains was only slightly different. *Arthrobacter terregens* and *Agr. ramosus* together with *Arthrobacter* sp. G109 (not included in Figs. 1 and 3) formed a loose group which joined cluster C at a level just below 75 % S. *Brevibacterium divaricatum* (which in Figs. 1 and 3 joined *Cellulomonas flavigena* in cluster D) also joined cluster C.

Cluster D (*Cellulomonas* and others). This group contained three subgroups (Figs. 2 and 4): D1 contained *Brevibacterium imperiale*, *Cellulomonas uda* and *Microbacterium lacticum* (2 strains); D2 contained *Corynebacterium mannii*, *Nocardia cellulans* and *N. turbata* (three strains); D3 contained *Cellulomonas biazotea*, *Cel. cellulasea*, *Cel. bibula*, *Cel. gelida*, *Cel. subalbus*, *Cel. flavigena* and *Arthrobacter* sp. G162. The composition of the groups was very similar in the other computer run (Figs. 1 and 3) even though *Nocardia cellulans*, *N. turbata* and *Arthrobacter* sp. G162 were not included in the run. The main difference was the grouping of *Br. divaricatum* with *Cel. flavigena* (cf. cluster C, Figs. 2 and 4).

Cluster E (*Propionibacterium* and others). At 80 % S three subgroups formed (Figs. 1 and 3). E1 contained all strains received as *Propionibacterium* spp. E2 contained *Listeria denitrificans* and three strains isolated as *Corynebacterium* spp. from poultry deep litter (Schefferle, 1966). E3 contained one strain received as *Corynebacterium* sp. and two received as *Arthrobacter* spp. No *Propionibacterium* spp. were included in the computer run illustrated in Figs. 2 and 4 but, with the exception of this omission, the composition was similar except for the grouping of *Brevibacterium incertum* (cluster C, Figs. 1 and 3) with *Corynebacterium* sp. (4C 1528).

Cluster F (*Arthrobacter*, *Brevibacterium*, *Kurthia*, *Mycobacterium*). This was a large cluster which contained 12 groups showing S values of 80 % or greater (Figs. 2 and 4). F1 contained seven *Kurthia* strains. *Corynebacterium mediolanum* (cf. cluster G, Figs. 1 and 3) joined this group at 77 % S. F2, with an overall similarity of 84 %, contained 14 strains received as *Arth. variabilis*, *Cor. fascians*, *Cor. rubrum*, *Corynebacterium* spp. and *Mycobacterium rhodochrous*. F3, with an overall similarity of 83 %, contained 20 strains received as *Arthrobacter* spp., *Cor. paurometabolum*, *Corynebacterium* spp., *Jensenia canicruria*, *Myco. rhodochrous*, *Nocardia calcarea* and *N. opaca*. Only six strains from subgroup F2 and seven strains from subgroup F3 were included in the computer run illustrated in Figs. 1 and 3. As can be seen, they merged to form one group (labelled F2) at 83 % S. Subgroup F4 contained six strains received as *Br. linens*, *Arthrobacter* spp. and *Corynebacterium* spp. (Figs. 2 and 4). However, in Figs. 1 and 3 the group has expanded with a slight drop in overall % S to include *Arth. simplex* and *Arth. crystallopoietes* (these strains grouped with other *Arthrobacter* spp. in subgroup F11; Figs. 2 and 4). F5 (Figs. 1 and 3) was a small group containing *Br. stationis*, *Br. ammoniogenes*, *Cor. equi* and a *Corynebacterium* sp. It joined subgroup F2 (Figs. 1 and 3) at 80 % S. In Figs. 2 and 4 the subgroup is represented by three strains only (*Cor. equi* was not included in this computer run) and joined subgroup F4 at about 83 % S.

Subgroup F6 (Figs. 2 and 4) contained 11 strains received as *Arth. duodecadis*, *Arthrobacter* spp. and *Cor. vesiculare*. The grouping of the strains in the other computer run (Figs. 1 and 3) was the same except that two strains, *Arthrobacter* spp. H72 and J30, were not included in this computation. Subgroup F7 (Figs. 2 and 4) was a small group containing only four strains (*Arth. flavescens*, *Cor. michiganense*, *Cor. rathayi* and *Micrococcus luteus* NCTC8512) with an overall similarity of 82 % S and joined subgroup F6 at 80 % S. In Figs. 1 and 3 the group has expanded to contain *Arth. citreus* which fell in subgroup F12 in Figs. 2 and 4. Subgroup F8 (Figs. 2 and 4) contained eight strains (87 % S) received as *Arth. aureus*, *Arth. citreus*, *Arth. simplex*, *Arth. variabilis*, *Arth. crystallopoietes*, *Arth. linens*, *Arth. stationis* and *Arth. ammoniogenes*.

Fig. 1. A sorted similarity matrix of the mainly facultatively anaerobic coryneforms.

Fig. 2. A sorted similarity matrix of the mainly aerobic coryneforms.

Arth. ureafaciens, *Arthrobacter* spp. and *Cor. ilicis*. The composition of the group was exactly the same in Figs. 1 and 3 except that *Arth. ureafaciens* M8916 was not included.

Subgroup F9 (Figs. 2 and 4) contained 13 strains (86 % S) received as *Arth. globiformis*, *Arth. pascens*, *Arth. ramosus* and *Arthrobacter* spp. The composition of the group is the same in Figs. 1 and 3 except for the three strains (M8911, G93 and H123) not included. Subgroup F10 (Figs. 2 and 4) contained five strains (85 % S) received as *Arth. atrocyaneus*, *Arth. oxydans*, *Cor. viscosum* and *Corynebacterium* spp. *Arthrobacter oxydans* NCIB9334 was not included in the computation illustrated in Figs. 1 and 3. Subgroup F11 (Figs. 2 and 4) contained eight strains (83 % S) received as *Arth. crystallopoietes*, *Arth. nicotianae*, *Arth. simplex*, *Arth. tumefaciens* and *Arthrobacter* spp. In Figs. 1 and 3 this subgroup is reduced to five strains (85 % S) because *Arth. simplex* NCIB8929 and *Arth. crystallopoietes* clustered in subgroup F4 and *Arth. simplex*; M8913 was not included. Subgroup F12 (Figs. 2 and 4) contained 11 strains (approx. 80 % S) received as *Arth. citreus*, *Arthrobacter* spp., *Cellulomonas fimi*, *Cor. aquaticum* and *Corynebacterium* spp. In Figs. 1 and 3 the subgroup is reduced to four strains because four strains were not included, *Cor. aquaticum* and *Corynebacterium* sp. 4D 9312 grouped in cluster G, and *Corynebacterium* sp. NCIB8191 clustered with *Arthrobacter* spp. G109 and J39 to form a small group labelled F13.

Cluster G (plant pathogenic corynebacteria). This was a small loose group containing six strains, mainly plant pathogenic *Corynebacterium* spp., with an overall similarity of 76 % (Figs. 1 and 3). In Figs. 2 and 4, three of the strains have joined subgroups in cluster F.

Cluster H (Figs. 2 and 4). This was a loose group containing 13 named *Nocardia* spp. and three named *Mycobacterium* spp. When the *Nocardia* spp. were not included (Figs. 1 and 3) the *Mycobacterium* spp. fell in cluster F, joining subgroup F5 at the 76 % S level.

Grouping of clusters

The eight main clusters grouped into three clear-cut larger areas. Area I containing clusters A and B had an overall similarity of about 74 %. Area II containing clusters C, D and E showed an overall similarity of 73 % and joined area I at 71 % S (Figs. 1 and 3). These two areas joined area III (overall similarity 70 % S) at approximately 65 % S (Figs. 2 and 4).

Characterization of groups and clusters

The ultimate aim of a study of this kind is to devise a system of practical identification. The salient reactions of the strains in the three major areas are listed in Table 2. The reactions of the strains in the eight clusters in these areas are depicted in Tables 3, 4, 5 and 6. Table 7 lists the characters which differentiate between *Microbacterium thermosphactum*, *Listeria* and *Erysipelothrix*.

Strain variation and test reproducibility

Strain variation. As noted in Methods, mucoid variants produced by two cultures, *Jensenia canicruria* NCIB8147 and *Myco. rhodochrous* G1257, were included in the study, labelled NCIB8147m and G1257m respectively. Additionally, duplicates of three strains of *Listeria* were received from sources other than N.C.T.C. Two were received from Dr N. S. Mair (Public Health Laboratory, Leicester) and are listed as NCTC4885 (Mair) and NCTC5105 (Mair). One was an old laboratory strain of NCTC7973 which had been maintained on solid medium for three years before the commencement of the study, and labelled NCTC7973a.

As can be seen from Figs. 2 and 4, there was little difference between the mucoid strains and the parent culture. In addition to the mucoid growth the difference was due to small variations in the degree of antibiotic sensitivity.

Table 2. *Salient characters of the three major areas as percentages of positive reactions*

	Area I* (Clusters A and B)	Area II* (Clusters C, D and E)	Area III† (Clusters F, G and H)
No. of strains tested	42	47	130
Colonial morphology			
Colonies pigmented	0	40	70
Mycelium formed	0	0	22
Blue/green iridescence (Henry technique)	45	0	0
Stained preparations			
Rods and filaments	50	4	5
Rods and cocci	0	49	78
Chains formed	100	34	9
Bipolar staining	0	28	1
Rods > 2 μ m long	0	26	38
Acid fastness	0	17	32
Growth at 37 °C	88	77	28
Anaerobic growth	100	76	37
Catalase	69	83	100
Benzidine	10	72	100
Oxidase	0	9	27
Acid from			
Arabinose	40	43	7
Rhamnose	74	19	12
Fructose	100	85	19
Galactose	95	68	8
Glucose (aerobic)	100	100	22
Melezitose	74	34	3
Raffinose	2	38	3
Salicin	88	45	8
Dulcitol	12	0	0
Glycerol	81	43	15
Hydrolysis tests			
Casein	5	34	47
Cellulose	0	9	0
Chitin	0	11	2
Gelatin	0	9	39
Tween 20 ppt	0	38	87
Tween 80 ppt	7	13	76
Tyrosine	0	13	65
Sole nitrogen source			
(NH ₄) ₂ SO ₄	0	30	82
KNO ₃	0	28	88
KNO ₃ + Casamino acid	38	57	95
Growth on 0.3 % (w/v) thallos acetate	100	13	13
Sensitivity‡			
Sulphonamide (H)	0	38	57
Fucidin (L)	45	100	85
Colomycin (L)	12	74	72
Methyl red	95	77	1
Voges Proskauer	83	17	5
Reduction NO ₃ → NO ₂	0	45	45
Production H ₂ S	0	19	88
Urease	0	26	61

* Information derived from material presented in Figs. 1 and 3.

† Information derived from material presented in Figs. 2 and 4 but *Cellulomonas rossica* and *Corynebacterium nephridii* not included.

‡ (L), low potency; (H), high potency.

Cluster

Area

Fig. 4. Dendrogram derived from Fig. 2 of the relationships between the mainly aerobic coryneforms.

Difference in antibiotic sensitivity was also the main reason for the variation noted between the three pairs of *Listeria* strains (Figs. 1 and 3). The only strain showing a large difference was NCTC7973a, which differed by about 8 % S from the more recently acquired strain. As the strains were all given study reference (C) numbers (listed in Table 1) and all bench work was done using the C numbers only, I was unaware of their true identity and thus bias was reduced to a minimum.

Test reproducibility. The effect of errors in microbiological tests on numerical taxonomic similarities has been discussed by Sneath & Johnson (1972). All tests were repeated twice on the same basal medium; in addition, some were repeated more frequently on the same medium, while others were repeated using a different basal medium or different conditions of incubation.

Some tests, e.g. degree of acid fastness and oxidase reaction, were bad tests in that very slight differences in basal media or technique gave different results; however, the reproducibility of the majority of the tests fell within the limits discussed by Sneath & Johnson (1972). The average percentage of discrepancies, *p*, was 2.4 % and therefore would introduce little distortion into the numerical analyses as noted by Sneath & Johnson (1972).

DISCUSSION

The composition of the three broad similarity areas, their relationship to each other and the taxonomic significance of the groupings will be discussed separately.

Area I

The grouping of the species *Microbacterium thermosphactum* and the genera *Listeria* and *Erysipelothrix* with representatives of the Lactobacillaceae confirms the evidence from previous numerical taxonomic studies (Davis *et al.* 1969; Stuart & Pease, 1972; Wilkinson, 1973) and that derived from enzyme and DNA base ratio studies (Collins-Thompson *et al.* 1972; Trivett & Meyer, 1971; Stuart & Welshimer, 1973; Flossman & Erler, 1972) that these bacteria show a closer relationship to the Lactobacillaceae than to the Corynebacteriaceae. The characteristics of the important taxa are given in Table 3.

Microbacterium thermosphactum. *Microbacterium thermosphactum* strains, together with the atypical lactobacilli of Thornley & Sharpe's (1959) group I, form a well defined cluster A1 (>90 % S) which joins the two *Lactobacillus* spp. at 83 % S. As pointed out by other workers (see Collins-Thompson *et al.* 1972) *Micro. thermosphactum* is quite different from the other species of the genus *Microbacterium* (see below), and on the basis of the present study is sufficiently distinct from a number of other closely allied genera to merit genus status.

Listeria. *Listeria monocytogenes* strains also form a distinct cluster, A3 (> 90 % S), joining the one strain of *List. grayi* at 87 % S and one strain (LTRS MT36) of Thornley & Sharpe's group III atypical lactobacilli at 84 % S, but quite distinct from both *Micro. thermosphactum* and *Erysipelothrix*. Because only one strain of *List. grayi* was included and no strains of *List. murrayi* were available at the start of this study, little can be said about the status of the latter two species or whether or not they are distinct enough from *List. monocytogenes* to be considered as separate species as suggested by Stuart & Welshimer (1973). The results based on the one strain of *List. grayi* indicate that this species is worthy of separate species status in the genus *Listeria*. This is in accord with the findings of Wilkinson (1973).

The association of the atypical *Lactobacillus* strain (LTRS MT36) with this group is

Table 3. *Characteristics of subgroups in clusters A and B as percentages of positive reactions*

Information derived from material presented in Figs. 1 and 3.

	<i>Micro. thermo-</i> <i>sphactum</i> A1	<i>Lactobacillus</i> A2	<i>Listeria</i> A3	<i>Strep. faecalis</i> A4	<i>Erysipelothrix</i> <i>Streptococcus</i> B
No. of strains tested	6	3	23	3	6
Colonial morphology					
Colony elevation flat	0	0	0	0	57
Colony elevation raised	17	0	4	0	0
Colony elevation convex	83	100	96	100	43
Colony elevation irregular	50	0	0	0	0
Colony mucoid	0	0	0	0	0
Colony leaves impression in agar	0	100	57	100	0
Colony margin entire	50	100	91	100	43
Colony margin irregular	50	0	9	0	57
Colony easily emulsified	100	100	100	100	100
Colony coarse suspension	0	0	0	0	0
Colony violet/blue	0	0	0	0	0
Colony pink/red	0	0	0	0	0
Colony yellow/orange/brown	0	0	0	0	0
Colony cream/grey/white	100	100	100	100	100
Exopigment, water-soluble	0	0	0	0	0
Crystals formed	0	0	0	0	0
Mycelium formed	0	0	0	0	0
Aerial hyphae	0	0	0	0	0
Blue/green iridescence (Henry technique)	0	0	83	0	0
Stained preparations					
Rods and filaments	83	33	43	0	71
Rods and cocci	0	0	0	0	0
Rods only	17	67	57	0	0
Branching present	0	0	0	0	0
Mycelium present	0	0	0	0	0
Cystites	0	0	0	0	0
Chains formed	100	100	100	100	100
Barred staining	67	100	96	0	71
Bipolar staining	0	0	0	0	0
Rods < 2 µm long	100	100	100	100	100
Rods > 2 µm long	0	0	0	0	0
Acid fastness	0	0	0	0	0
Homogeneous growth in SEB	100	100	100	100	29
Collar/pellicle in SEB	0	0	0	0	0
Growth on blood agar	100	100	100	100	100
β-Haemolysis	0	0	91	0	17
Greening of blood	100	100	4	0	0
Motility	0	0	83	100	0
Microcolonies	0	0	0	13	14
Flagella	0	0	70	67	0
Growth at					
4 °C	100	100	100	100	0
10 °C	83	100	100	100	71
25 °C	100	100	100	100	100
30 °C	100	100	100	100	100
37 °C	17	100	100	100	100
45 °C	17	33	4	100	0
50 °C	0	0	0	0	0
Survival at 60 °C for 30 min	0	0	0	100	0
Anaerobic growth	100	100	100	100	100

Table 3 (cont.)

	<i>Micro. thermo- sphactum</i>	<i>Lactobacillus</i>	<i>Listeria</i>	<i>Strep. faecalis</i>	<i>Erysipelothrix Streptococcus</i>
	A1	A2	A3	A4	B
Catalase	100	0	91	67	0
Benzidine	17	0	13	0	0
Oxidase	0	0	0	0	0
Acid from					
Arabinose	100	100	0	100	86
Xylose	100	100	0	100	14
Rhamnose	83	67	91	67	14
Fructose	100	100	100	100	100
Galactose	100	100	87	100	100
Glucose					
Aerobic	100	100	100	100	100
Anaerobic	100	100	100	100	100
Mannose	100	100	100	100	100
Cellobiose	100	100	100	100	29
Lactose	100	100	96	100	100
Sucrose	100	100	96	100	29
Maltose	100	100	100	100	100
Melezitose	100	100	74	100	29
Raffinose	17	67	0	0	0
Melibiose	100	100	0	0	14
Sorbose	0	33	0	33	14
Salicin	100	100	100	100	14
Adonitol	100	100	0	100	14
Inositol	100	67	9	67	0
Dulcitol	83	0	0	0	0
Mannitol	100	100	4	100	14
Glycerol	100	67	100	100	0
Sorbitol	100	100	13	100	14
Hydrolysis tests					
Aesculin blackening	83	100	100	100	14
Aesculin loss purple fluores-					
cence	67	100	100	100	29
Arginine	16	33	87	100	57
Arginine (Thornley)	0	33	9	100	57
Casein	0	0	13	67	29
Cellulose	0	0	0	0	0
Chitin	0	0	0	0	0
Gelatin	0	0	0	0	0
DNA	0	0	0	0	29
Hippurate	0	67	91	100	57
Tween 20 clearing	0	0	4	0	0
Tween 20 ppt	0	0	0	0	0
Tween 40 clearing	0	33	9	0	0
Tween 40 ppt	0	0	83	100	0
Tween 60 clearing	0	0	0	67	0
Tween 60 ppt	17	67	100	100	29
Tween 80 clearing	0	0	0	0	0
Tween 80 ppt	0	0	13	0	0
Tyrosine	0	0	0	0	0
Pigment from tyrosine	0	0	0	0	0
Xanthine	0	0	0	0	0
Starch	0	0	0	0	0
Starch (in 10 % CO ₂)	0	0	0	0	0
Sole carbon source					
Citrate	0	0	0	100	0
Gluconate	17	100	100	100	0
Glucose	100	100	100	100	100

Table 3 (*cont.*)

	<i>Micro. thermo- sphaetum</i> A1	<i>Lactobacillus</i> A2	<i>Listeria</i> A3	<i>Strep. faecalis</i> A4	<i>Erysipelothrix Streptococcus</i> B
Sole carbon source (<i>cont.</i>)					
Hippurate	0	0	0	0	0
Malate	0	0	0	100	0
Propionate	0	0	0	0	0
Pyruvate	0	0	4	100	0
Succinate	0	0	0	0	0
Sole nitrogen source					
(NH ₄) ₂ SO ₄	0	0	0	0	0
KNO ₃	0	0	0	0	0
KNO ₃ + Casamino acid	0	33	57	100	0
Growth in presence of					
NaCl					
5 % (w/v)	100	0	100	100	29
10 % (w/v)	83	33	87	0	0
Thallous acetate					
0.01 % (w/v)	100	100	100	100	100
0.02 % (w/v)	100	100	100	100	86
0.03 % (w/v)	100	100	100	100	86
Sodium azide					
0.01 % (w/v)	0	67	26	100	100
0.02 % (w/v)	0	67	4	100	43
0.05 % (w/v)	0	67	4	100	43
Potassium thiocyanate					
2.5 % (w/v)	83	33	96	100	14
3.75 % (w/v)	16	33	96	33	0
4.5 % (w/v)	0	33	96	33	0
Tetrazolium					
0.01 % (w/v)	100	100	100	100	71
Reduction 0.01 % (w/v)	50	0	0	67	0
0.1 % (w/v)	0	100	91	100	0
Reduction 0.1 % (w/v)	0	33	87	100	0
Potassium tellurite					
0.01 % (w/v)	0	33	96	100	100
Reduction 0.01 % (w/v)	0	33	96	100	100
0.025 % (w/v)	0	0	87	100	100
Reduction 0.025 % (w/v)	0	0	87	100	100
0.05 % (w/v)	0	0	87	100	86
Reduction 0.05 % (w/v)	0	0	87	100	86
Lysis by lysozyme	100	33	100	100	0
Lysis by lysozyme + EDTA	100	33	100	100	0
Sensitivity to*					
Penicillin (H)	100	67	100	100	100
Streptomycin (H)	0	100	100	100	71
Chloramphenicol (H)	100	100	100	100	100
Aureomycin (H)	100	100	96	100	100
Terramycin (H)	100	100	96	100	86
Erythromycin (H)	100	100	100	100	86
Tetracycline (H)	100	100	96	100	100
Sulphonamide (H)	0	0	0	0	0
Ledermycin (H)	100	100	100	100	86
Celbenin	0	0	43	0	86
Kanamycin (H)	100	67	100	33	0
Penbritin (H)	100	100	100	100	86
Spiramycin (L)	100	100	100	33	71
Vancomycin (L)	100	33	100	100	57
Paromomycin (H)	100	33	100	100	0

* (H), high potency; (L), low potency.

Table 3 (cont.)

	<i>Micro. thermo- sphactum</i>	<i>Lactobacillus</i>	<i>Listeria</i>	<i>Strep. faecalis</i>	<i>Erysipelothrix Streptococcus</i>
	A1	A2	A3	A4	B
Sensitivity to (cont.)					
Fucidin (L)	100	0	30	0	86
Neomycin (H)	100	67	100	67	14
Bacitracin	100	100	100	100	100
Novobiocin (H)	100	100	100	100	29
Oleandomycin (H)	100	100	100	100	71
Colomycin (L)	67	0	4	0	0
Polymyxin (L)	100	67	100	0	29
Nitrofurantoin	100	67	87	33	43
Methyl red	100	100	100	100	71
Voges Proskauer	100	100	100	100	0
Egg yolk ppt	0	67	61	33	14
Egg yolk clearing	0	0	0	0	0
Litmus milk					
Acid production	0	100	96	100	100
Alkaline production	0	0	0	0	0
Coagulation	0	67	0	100	0
Digestion	0	0	0	67	0
Peptonization	0	0	0	0	0
Reduction	0	33	9	100	29
Reduction NO ₃ → NO ₂	0	0	0	0	0
Reduction NO ₃ → N ₂	0	0	0	0	0
Reduction NO ₃ beyond N ₂	0	0	0	0	0
Production H ₂ S	0	0	0	0	0
Urease	0	0	0	0	0
Sulphatase	0	0	0	0	0
Phosphatase	100	100	96	100	29
Ammonia from SEB	0	0	0	0	0

interesting, but no serological relationships could be detected between this strain and antisera prepared against *List. monocytogenes*, *List. grayi* or *List. murrayi*.

As noted by Stuart & Welshimer (1973) *List. denitrificans* is quite different from the other members of the genus and in the present study it groups loosely with some *Corynebacterium* strains isolated from poultry and *Arthrobacter* spp. (A137/68 and Brzin) isolated from the human throat. This cluster may well form the basis of an as yet unrecognized taxon.

Erysipelothrix. The four recently isolated strains of *E. rhusiopathiae* grouped in a tight cluster at about 93 % S but the strain NCTC8163 showed a number of differences which resulted in an apparently closer similarity of this strain to a *Streptococcus* sp. of serological group G. Of interest is the association of *Strep. faecalis* strains with the *Lactobacillus*–*Listeria*–*Micro. thermosphactum* group, while the one strain of *Strep. pyogenes* and the *Streptococcus* strain of serological group G showed a closer association with the *Erysipelothrix* cluster. A similar close association between *Erysipelothrix* and *Streptococcus* was noted by Davis *et al.* (1969), but these authors doubted the validity of their results because of the limitations imposed when a variety of different bacteria are studied under one set of conditions. Although the % guanine–cytosine (GC) base ratio of *Erysipelothrix* (36 to 38; Flossmann & Erler, 1972) is in the same range as that of the streptococci, the cell walls of *Erysipelothrix* contain diaminopimelic acid which has never been detected in the cell walls of streptococci.

Classically, the presence of cytochromes and catalase have excluded bacteria from the Lactobacillaceae, but it is now recognized that some *Lactobacillus* species are catalase

positive (Dacre & Sharpe, 1956; Vankova, 1957; Whittenbury, 1964) and in our experience *Listeria* strains which are normally strongly catalase positive when grown on rich laboratory media are catalase negative if grown on a medium low in yeast extract although they grow equally well. We were, however, able spectrophotometrically to demonstrate low levels of cytochromes in *List. monocytogenes* (Jones, unpublished) though Trivett & Meyer (1971) reported the absence of cytochromes in *Listeria*. *Microbacterium thermosphactum*, although catalase positive, frequently gives an equivocal benzidine reaction. However, this is also dependent on the composition of the growth medium, and using spectrophotometric techniques Davidson, Mobbs & Stubbs (1968) demonstrated a full cytochrome system in these bacteria. No catalase or cytochromes have been detected in *Erysipelothrix*.

A strong case can be made for the inclusion of *Erysipelothrix*, *Listeria* and *Micro. thermosphactum* in the family Lactobacillaceae. It can be argued that the inclusion of these taxa in this family makes an already large family unwieldy. The alternative is to create a new family. However, this seems premature because there has been no comprehensive comparison of *Erysipelothrix*, *Listeria*, *Micro. thermosphactum* and the interesting atypical lactobacilli of Thornley & Sharpe (1959) with all the genera presently included in the Lactobacillaceae. Such a study should be performed before any 'family of convenience' is created to accommodate refugees from other bacterial families. The study of Wilkinson (1973), which covers a great deal but not all of this area, is in accord with this latter view.

Area II

This area consists of three main phenons C, D and E (Figs. 1 and 3). Of the eight subgroups, four can be equated with the established genera *Corynebacterium*, *Cellulomonas*, *Microbacterium* and *Propionibacterium*, two are centred around the species *Cor. pyogenes* and *List. denitrificans*, one contains the cellulolytic nocardias, and one the 'throat' arthrobacters.

As can be seen from Table 2, there is no one character or set of characters which distinguishes area I from area II. There are catalase- and cytochrome-containing strains in both areas, although they predominate in area II. A character which seems potentially useful for the characterization of the areas is the mode of division of the bacteria. Chain formation is less common in area II, whereas the snapping type of cell division characteristic of area II, resulting in the production of V and Chinese-letter forms, is not typical of area I. However, in our experience this character is influenced by the mode of culture and in a routine laboratory where an unknown bacterium would be grown on solid routine laboratory media a strain of *Lactobacillus* can exhibit typical V forms (and see *Streptobacterium plantarum* No. 5 of Orla-Jensen, 1919; Plate XLIII). The characteristics of the important taxa are given in Table 4.

Corynebacterium. There is general if not universal agreement that the genus *Corynebacterium* includes the type species *Cor. diphtheriae* and its close relatives (Jensen, 1952; Barksdale, 1970; Harrington, 1966; Yamada & Komagata, 1972*b*). To this 'animal group' have been added various other species, e.g. *Cor. equi*, *Cor. pyogenes* and *Cor. fascians*, but doubts have been expressed on whether some or any of them belong to the genus (Jensen, 1952; Barksdale, 1970; Lelliott, 1966; Robinson, 1966*a*). On the other hand there is a growing opinion that the species *Micro. flavum* should be reclassified in the genus *Corynebacterium* (Collins-Thompson *et al.* 1972). The grouping of *Cor. diphtheriae* and the other animal species plus *Micro. flavum* in subgroup C1 of the present study is in keeping with the reported % GC base ratios (Bouisset, Breuillard & Michel, 1963; Bousfield, 1972; Collins-Thompson *et al.* 1972). Support also comes from the numerical taxonomic study of Harrington (1966) who found a group containing several animal coryneforms, but *Cor. murium* was related

at a lower % S value, as were *Cor. pyogenes* and *Cor. haemolyticum*. The close association of *Cor. ovis* with *Cor. diphtheriae* is in accord with the report of Barksdale (1970) that when lysogenized with a *tox*⁺ phage *Cor. ovis* synthesizes diphtheria toxin. However, Barksdale (1970) suggested the retention of all the species grouped in subgroup C₁ of the present study, plus *Cor. equi*, in the genus *Corynebacterium*, and Yamada & Komogata (1972*b*) also included *Cor. equi* and *Cor. fascians* in the genus. This is the opposite of the results of the present study, where *Cor. equi* and *Cor. fascians* showed a relationship to the '*Mycobacterium rhodochrous*' group. Additionally, both these species contain a lipid constituent which Goodfellow, Minnikin, Patel & Modarska (1973) consider to be characteristic of the '*Myco. rhodochrous*' complex.

Microbacterium flavum. The association of *Micro. flavum* with the animal corynebacteria is in agreement with data from numerical studies (Bousfield, 1972; Stuart & Pease, 1972), cell wall studies (Cummins & Harris, 1956; Robinson, 1966*b, c*; Schleifer, 1970), electrophoretic patterns of catalases and esterases (Robinson, 1966*a, c*; Collins-Thompson *et al.* 1972), and metabolic pathways and % GC DNA[†]base ratio (Collins-Thompson *et al.* 1972).

Microbacterium lacticum – *Brevibacterium imperiale*. *Microbacterium lacticum*, the type species of the genus *Microbacterium*, is far more closely related to *Micro. flavum* than either are to *Micro. thermosphactum*. Indeed, there have been a number of suggestions that *Micro. lacticum* should be reclassified as *Corynebacterium* (Abd-el-Malek & Gibson, 1952; Jensen, 1932; Collins-Thompson *et al.* 1972). However, in the present study *Micro. lacticum* clusters with *Br. imperiale* (D₁). This separation from the animal corynebacteria is in keeping with the reported % GC DNA base ratios of 63 (Collins-Thompson *et al.* 1972) and 69.3 (Yamada & Komogata, 1970*b*), with biochemical and serological studies (Robinson, 1966*b, c*), and with the numerical taxonomic studies of Davis *et al.* (1969), Stuart & Pease (1972) and Bousfield (1972).

The close association between *Micro. lacticum* and *Br. imperiale* noted in Figs. 1 and 3 is in keeping with the results of Yamada & Komagata (1972*b*). It is possible that this small cluster is synonymous with the genus *Microbacterium* (Orla-Jensen, 1919) and the proposal to omit the genus from the forthcoming edition of Bergey's Manual (Collins-Thompson *et al.* 1972) is premature.

Corynebacterium pyogenes. The generic position of *Cor. pyogenes* has been questioned for some time. A number of workers have presented evidence of a close affinity to the streptococci (Barksdale, Li, Cummins & Harris, 1957; Sneath & Cowan, 1958; Barksdale, 1970). Stuart & Pease (1972), in their computation of similarity between coryneform bacteria based on 'general characters', also found a close association between *Cor. pyogenes* and streptococci, but in the same study the computation of similarity based on physiological characteristics indicated a closer relationship to the animal corynebacteria. Bousfield (1972) noted a relationship between *Cor. pyogenes* and *Listeria* and *Erysipelothrix*, all of which showed some relationship to the animal corynebacteria. Harrington (1966) noted that *Cor. pyogenes* and *Cor. haemolyticum* (which is probably a haemolytic variant of *Cor. pyogenes*; Barksdale *et al.* 1957) clustered together and were quite distinct from the main group of animal corynebacteria.

In the present study the two strains of *Cor. pyogenes* differed slightly (Figs. 1 and 3), one showing a closer relationship to *Br. incertum* (which is haemolytic), the other showing a closer relationship to *Cor. haemolyticum*. *Corynebacterium pyogenes* is probably a variable species containing several subgroups (Roberts, 1968).

'*Bacterium eurydice*', which clusters with the *Cor. pyogenes* group, is an interesting bacterium whose relationship, if any, to *Strep. pluton* is not clear (Bailey, 1957, 1960, 1963).

Table 4. *Characteristics of subgroups in clusters C, D and E as percentages of positive reactions*

	<i>Cor. diphtheriae and related bacteria</i> C1*	<i>Cor. pyogenes and related bacteria</i> C2*†	<i>Micro. lacticum Br. imperiale</i> D1*	<i>N. cellulans N. turbata</i> D2‡	<i>Cellulo- monas</i> D3*	<i>Propioni- bacteria</i> E1*	<i>List. denitrifi- cans and coryne- forms</i> E2 and E3*
No. of strains tested	13	8	3	5	9	5	7
Colonial morphology							
Colony elevation flat	0	0	0	0	0	0	29
Colony elevation raised	0	25	0	0	11	0	14
Colony elevation convex	100	75	100	100	89	100	57
Colony elevation irregular	0	0	0	0	0	0	0
Colony mucoid	0	0	0	0	11	0	0
Colony leaves impres- sion in agar	54	38	33	100	44	40	14
Colony margin entire	100	100	100	20	100	100	71
Colony margin irregular	0	0	0	80	0	0	29
Colony easily emulsified	92	100	100	100	100	80	71
Colony coarse suspen- sion	8	0	0	0	0	20	29
Colony violet/blue	0	0	0	0	0	0	0
Colony pink/red	0	0	0	0	0	0	0
Colony yellow/orange/ brown	15	0	66	100	100	20	43
Colony cream/grey/ white	85	100	33	0	0	80	57
Exopigment, water soluble	8	0	0	0	22	0	0
Crystals formed	0	0	0	0	0	0	0
Mycelium formed	0	0	0	0	0	0	0
Aerial hyphae	0	0	0	0	0	0	0
Blue/green iridescence (Henry technique)	0	0	0	0	0	0	0
Stained preparations							
Rods and filaments	0	0	0	0	0	0	29
Rods and cocci	15	25	33	100	56	100	86
Rods only	85	75	66	0	44	0	14
Branching present	0	0	0	80	56	40	14
Mycelium present	0	0	0	60	0	0	0
Cystites	0	0	0	0	0	40	57
Chains formed	31	75	66	0	11	20	29
Barred staining	23	50	33	100	89	100	86
Bipolar staining	69	38	33	0	0	0	0
Rods < 2 µm long	100	100	100	80	44	40	43
Rods > 2 µm long	0	0	0	20	56	60	57
Acid fastness	0	13	0	60	11	0	29
Homogeneous growth in SEB	92	100	100	100	100	80	100
Collar/pellicle in SEB	31	0	0	0	22	0	29
Growth on blood agar	100	100	100	100	100	100	100
β-Haemolysis	23	25	0	0	0	0	0
Greening of blood	8	0	0	60	0	0	57
Motility	0	13	33	20	67	0	14
Microcolonies	0	0	0	0	0	0	0
Flagella	0	0	33	0	22	0	14

* From material presented in Figs. 1 and 3.

† *Agromyces ramosus* and *Arthrobacter terregens* not included.

‡ From material presented in Figs. 2 and 4.

Table 4 (cont.)

	<i>Cor. diphtheriae and related bacteria</i> C1*	<i>Cor. pyogenes and related bacteria</i> C2*†	<i>Micro. lacticum Br. imperiale</i> D1*	<i>N. cellulans N. turbata</i> D2‡	<i>Cellulo- monas</i> D3*	<i>Propioni- bacteria</i> E1*	<i>List. dentrificans and coryne- forms</i> E2 and E3*
Growth at							
4 °C	8	13	100	100	44	60	43
10 °C	23	13	100	100	89	60	57
25 °C	100	88	100	100	100	100	100
30 °C	100	100	100	100	100	100	100
37 °C	85	100	100	20	67	100	43
45 °C	0	0	0	0	0	0	0
50 °C	0	0	0	0	0	0	0
Survival at 60 °C for							
30 min	0	0	66	80	11	0	0
Anaerobic growth	85	50	33	80	56	100	100
Catalase	100	13	100	100	100	60	100
Benzidine	100	13	100	100	89	100	57
Oxidase	18	0	0	40	22	0	0
Acid from							
Arabinose	0	13	33	100	89	100	57
Xylose	8	25	33	100	89	100	57
Rhamnose	8	13	0	60	22	40	43
Fructose	92	50	100	40	100	100	100
Galactose	46	63	100	100	78	100	86
Glucose							
Aerobic	100	100	100	100	100	100	100
Anaerobic	92	100	100	100	100	100	100
Mannose	77	50	100	60	89	100	100
Cellobiose	8	25	66	100	89	60	71
Lactose	8	38	66	60	56	80	57
Sucrose	8	50	66	100	100	100	86
Maltose	69	88	100	60	100	100	100
Melezitose	8	50	0	60	33	100	43
Raffinose	8	13	33	40	22	40	14
Melibiose	8	38	0	20	33	100	57
Sorbose	0	0	0	0	0	0	0
Salicin	0	13	100	100	100	60	57
Adonitol	15	0	0	0	0	100	43
Inositol	0	0	0	0	11	100	0
Dulcitol	0	0	0	0	0	0	0
Mannitol	8	25	66	0	22	60	71
Glycerol	23	0	33	80	44	100	100
Sorbitol	15	0	66	0	11	100	29
Hydrolysis tests							
Aesculin blackening	8	25	100	100	89	100	100
Aesculin loss purple fluorescence	8	25	33	100	89	100	100
Arginine	77	13	100	100	100	100	71
Arginine (Thornley)	15	13	33	0	11	0	29
Casein	15	38	66	80	78	0	29
Cellulose	0	0	0	20	44	0	0
Chitin	0	0	0	100	56	0	0
Gelatin	0	0	33	0	22	0	14
DNA	46	0	100	100	100	0	57
Hippurate	46	25	0	20	11	20	14
Tween 20 clearing	8	0	0	20	0	0	14
Tween 20 ppt	85	13	0	0	33	0	29
Tween 40 clearing	0	0	66	80	44	20	57
Tween 40 ppt	92	50	33	20	78	40	0

Table 4 (cont.)

	<i>Cor. diphtheriae and related bacteria</i> C1*	<i>Cor. pyogenes and related bacteria</i> C2*†	<i>Micro. lacticum Br. imperiale</i> D1*	<i>N. cellulans N. turbata</i> D2‡	<i>Cellulo- monas</i> D3*	<i>Propioni- bacteria</i> E1*	<i>List. denitrifi- cans and coryne- forms</i> E2 and E3*
Hydrolysis test (cont.)							
Tween 60 clearing	0	0	100	100	89	40	14
Tween 60 ppt	100	63	33	100	89	40	29
Tween 80 clearing	23	0	0	0	0	0	14
Tween 80 ppt	85	13	0	40	89	80	0
Tyrosine	23	13	66	0	0	0	0
Pigment from tyrosine	0	13	66	0	0	0	0
Xanthine	0	0	0	40	11	0	0
Starch	23	13	66	100	89	20	100
Starch (in 10 % CO ₂)	31	25	66	100	78	40	43
Sole carbon source							
Citrate	15	0	33	80	33	0	0
Gluconate	54	38	66	100	89	60	14
Glucose	69	38	100	100	100	100	14
Hippurate	0	0	0	0	0	0	0
Malate	69	0	33	80	67	0	14
Propionate	15	0	0	0	0	0	0
Pyruvate	38	13	100	80	67	60	14
Succinate	38	0	0	0	44	0	14
Sole nitrogen source							
(NH ₄) ₂ SO ₄	15	0	66	100	89	20	0
KNO ₃	8	0	100	100	78	20	0
KNO ₃ + Casamino acid	54	13	100	100	100	60	43
Growth in presence of							
NaCl							
5 % (w/v)	100	0	33	100	67	100	86
10 % (w/v)	46	0	0	40	11	40	29
Thallous acetate							
0.01 % (w/v)	31	38	33	0	11	0	29
0.02 % (w/v)	0	38	33	0	0	0	29
0.03 % (w/v)	0	38	33	0	0	0	29
Sodium azide							
0.01 % (w/v)	46	25	0	0	0	100	0
0.02 % (w/v)	8	25	0	0	0	100	0
0.05 % (w/v)	0	0	0	0	0	20	0
Potassium thiocyanate							
2.5 % (w/v)	92	25	66	100	78	100	100
3.75 % (w/v)	62	13	33	100	33	60	100
4.5 % (w/v)	46	0	0	100	11	0	57
Tetrazolium							
0.01 % (w/v)	62	38	0	100	22	0	100
Reduction 0.01 % (w/v)	18	0	0	40	22	0	57
0.1 % (w/v)	0	0	0	0	0	0	0
Reduction 0.1 % (w/v)	0	0	0	0	0	0	0
Potassium tellurite							
0.01 % (w/v)	100	0	0	40	89	0	43
Reduction 0.01 % (w/v)	100	0	0	40	89	0	43
0.025 % (w/v)	77	0	0	20	67	0	29
Reduction 0.025 % (w/v)	77	0	0	20	67	0	29
0.05 % (w/v)	62	0	0	0	44	0	29
Reduction 0.05 % (w/v)	62	0	0	0	44	0	29
Lysis by lysozyme	0	63	33	100	100	100	100
Lysis by lysozyme + EDTA	0	63	33	100	100	100	100
Sensitivity to §							
Penicillin (H)	100	100	100	40	89	100	86

§ (H), high potency; (L) low potency.

Table 4 (cont.)

	<i>Cor.</i> <i>diphtheriae</i> and related bacteria C1*	<i>Cor.</i> <i>pyogenes</i> and related bacteria C2*†	<i>Micro.</i> <i>lacticum</i> <i>Br.</i> <i>imperiale</i> D1*	<i>N. cellulans</i> <i>N. turbata</i> D2‡	<i>Cellulo-</i> <i>monas</i> D3*	<i>Propioni-</i> <i>bacteria</i> E1*	<i>List.</i> <i>denitrifi-</i> <i>cans</i> and coryne- forms E2 and E3*
Sensitivity to (cont.)							
Streptomycin (H)	100	100	100	100	100	100	100
Chloramphenicol (H)	100	100	100	100	100	80	100
Aureomycin (H)	100	88	100	100	100	100	100
Terramycin (H)	100	75	100	100	100	100	100
Erythromycin (H)	100	63	100	100	100	80	100
Tetracycline (H)	100	50	100	100	100	100	100
Sulphonamide (H)	31	38	0	100	100	40	0
Ledermycin (H)	100	100	100	100	100	100	100
Celbenin	92	75	100	0	22	40	57
Kanamycin (H)	100	88	100	100	56	100	100
Penbritin (H)	92	88	66	100	100	100	100
Spiramycin (L)	92	88	100	100	100	100	100
Vancomycin (L)	100	100	100	100	100	100	100
Paromomycin (H)	92	88	66	100	44	80	100
Fucidin (L)	100	100	100	100	100	100	100
Neomycin (H)	100	75	100	100	100	60	86
Bacitracin	100	100	100	100	100	80	100
Novobiocin (H)	92	88	100	100	100	80	100
Oleandomycin (H)	100	88	100	100	100	100	100
Colomycin (L)	54	75	66	100	89	60	100
Polymyxin (L)	92	75	100	100	100	100	100
Nitrofurantoin	92	100	33	20	67	80	100
Methyl red	69	88	100	100	89	100	57
Voges Proskauer	23	0	33	60	0	40	29
Egg yolk ppt	38	50	0	0	11	0	0
Egg yolk clearing	15	0	0	0	33	0	0
Litmus milk							
Acid production	23	25	100	100	78	100	43
Alkaline production	61	38	0	0	22	0	14
Coagulation	0	25	0	0	0	40	0
Digestion	0	13	0	0	0	40	0
Peptonization	8	13	0	0	11	0	0
Reduction	8	0	100	100	22	20	29
Reduction NO ₃ → NO ₂	31	13	33	100	89	20	71
Reduction NO ₃ → N ₂	0	0	0	0	0	0	0
Reduction NO ₃ beyond N ₂	0	0	0	0	0	0	0
Production H ₂ S	38	0	0	0	11	60	0
Urease	30	13	0	40	67	0	14
Sulphatase	0	0	0	0	0	0	0
Phosphatase	77	50	100	100	78	60	14
Ammonia from SEB	69	25	0	0	11	0	0

It is isolated from foul brood disease in bees and may well be related to some of the so-called *Brevibacterium* spp. isolated by Steinhaus (1941) and Lysenko (1959) from insects. The close association of *Br. incertum* with this group is in keeping with this hypothesis. However, the appearance of *Cor. murium* in this loose cluster is perplexing. Its % GC DNA base ratio (58.5) is different from the values of 46 to 48 shown by strains of *Cor. pyogenes*.

The loose association of *Arth. terregens* (% GC, 68.7; Skyring & Quadling, 1970) and *Agr. ramosus* with this group throws little light on their true taxonomic position. It does however indicate that they should be removed from the genus *Arthrobacter*.

Nocardia turbata – *Nocardia cellulans*. The close grouping of all strains of *N. turbata* and

their relationship to *N. cellulans* and *Cor. manihot* is in agreement with the suggestion that these strains do not belong to the genus *Nocardia* (LeChevalier & LeChevalier, 1970; Jones & Bradley, 1964). Prauser, LeChevalier & LeChevalier (1970) have proposed that *N. turbata* strains be separated as a new genus *Oerskovia* in the Actinomycetales. Jones & Bradley (1964), however, suggested that they should be included in the genus *Cellulomonas*. The degree of their relationship to *Cellulomonas* in the present study is probably that of a different genus rather than a species within a genus. However, the *N. turbata*-*N. cellulans* group falls quite clearly in area II of this study rather than area III which includes the other *Nocardia* spp.

Cellulomonas. This genus was rationalized by Clark (1951, 1952, 1953) and presently contains ten species (*Bergey's Manual of Determinative Bacteriology*, 1957). In the present study all representatives grouped as a distinct cluster with the exception of *Cel. fimi* and *Cel. rossica*. The Gram reaction of all the species has been inconsistent (Clark, 1951, 1953). In our hands *Cel. rossica* proved to be Gram-negative but with faint traces of Gram-positive staining (as did a number of soil corynebacteria) but the mode of regulation of its citrate synthase (Jones & Weitzman, 1971) was clearly of the Gram-negative pattern (P. D. J. Weitzman and D. Jones, unpublished).

Doubts have also been expressed on the inclusion of *Cel. fimi* in the genus *Cellulomonas* (Jensen, 1934). In the study of Bousfield (1972) *Cel. fimi* clustered with some Gram-positive 'Flavobacteria'. The results of the present study indicate that it should be reclassified in the genus *Arthrobacter*.

The main *Cellulomonas* cluster (D3, Figs. 1 and 3) merits genus rank quite distinct from the genus *Arthrobacter*, a conclusion also reached by Yamada & Komagata (1972*b*) on quite different criteria.

Propionibacterium. The close clustering of *Propionibacterium* species is in agreement with previous numerical studies (Seyfried, 1968; Malik, Reinbold & Vedamuthu, 1968). The intragroup relationships are in good accord with both these previous studies and also with the DNA homology studies of Johnson & Cummins (1972) which showed *P. shermanii* and *P. freudenreichii* to be in the same DNA homology group and *P. petersonii* and *P. jensenii* to be in another one. However, in the present study *P. pentosaceum* shows a close relationship to *P. petersonii* although it fell into a third DNA homology group (Johnson & Cummins, 1972). This apparent discrepancy is probably the result of the small number of *Propionibacterium* strains included here.

The lack of any close relationship between *Propionibacterium* and *Lactobacillus* confirms the results of Seyfried (1968).

Area III

Area III is the most heterogeneous of the three major groupings. It contains *Arthrobacter*, some species of *Brevibacterium*, *Kurthia*, and also extends into the Actinomycetales with the inclusion of *Mycobacterium* and *Nocardia*. The characteristics of the important taxa are given in Tables 5 and 6.

Arthrobacter. The genus *Arthrobacter* was created by Conn & Dimmick (1947) around the type species of *Arth. globiformis* to accommodate the soil coryneforms which exhibited a rod-coccus 'life cycle'. This life cycle is not confined to *Arthrobacter* spp., as has also been noted by other workers (see Bousfield, 1972). However, with one or two exceptions all strains received as *Arthrobacter* clustered with strains received as *Cel. fimi* and with various named *Corynebacterium* spp. to form one rather loose taxon containing several distinct groupings (see group F, Figs. 1, 2, 3 and 4).

The relationship between *Arth. globiformis*, *Arth. pascens*, *Arth. ramosus* and all the strains

included as representatives of 'nutrient group A' of Keddle *et al.* (1966) is in good accord with the classification of these workers based on nutritional requirements.

All representatives of 'nutritional group B' of Keddle *et al.* (1966), two of their 'nutritional group D' strains, plus nutritional group-intermediate C clustered with *Arth. duodecadis*, placed by Keddle *et al.* (1966) in the nutritionally 'ungrouped strains'.

The relationship between *Arth. ureafaciens*, *Arth. aurescens* and *Cor. ilicis* is in agreement with the close association of the latter two species noted by Bousfield (1972) and with the relationship between the first two species noted by Masuo & Nakagawa (1969) and Davis & Newton (1969).

Of the remaining *Arthrobacter* strains, *Arth. oxydans* appeared distinct and worthy of species status as did *Arth. simplex*, though the two representatives of the latter species showed a close association with *Arth. crystallopoietes*. Other clusters which probably merit species status are those groups of strains clustering around *Arth. atrocyaneus*: *Arth. citreus*, *Arth. tumefaciens*, *Arth. nicotianaeae*, *Cel. fimi*, *Cor. michiganense* and *Cor. rathayi*. The last three bacteria should be reclassified as *Arthrobacter* spp.

Kurthia. The division of *Kurthia* strains into two clusters, albeit with a high inter-similarity, is in keeping with the reports of Keddle (1943) and Gardner (1969). Four of the strains in this study were also used by Gardner. *Kurthia zopfii* (NCIB8603), of Gardner's group I, clustered with K2, S8 and S9, while the representatives of Gardner's group II clustered as a separate group. All seven strains of *K. zopfii* produced H₂S from cysteine, though they are reported not to do so from peptone water (Keddle, 1949) and SIM Difco medium (Gardner, 1969).

The taxonomic position of *Kurthia* is problematical. Although clearly in area III, *Kurthia* was not closely allied to any of the other genera in this area. It has been isolated mainly from milk and meat (see Gardner, 1969) but Keddle (1949) is of the opinion that it is a soil bacterium. There may well be a number of soil bacteria related to *Kurthia* which have not yet been studied in detail. As stressed by Gardner (1969), the colonial morphology of *Kurthia* strains resembles that of *Micro. thermosphactum*. However, the physiological and biochemical reactions of these two taxa are quite different and the apparent relationship between the two noted by Davis & Newton (1969) was due to the omission from their study of most of the physiological tests. The later study of Davis *et al.* (1969), which included a broad range of tests, did not show any close affinity between them.

Mycobacterium rhodochrous. The results are in complete agreement with those of other numerical taxonomic studies (Goodfellow, 1971; Goodfellow *et al.* 1972) which indicate that *Myco. rhodochrous*, along with a variety of other species, forms a recognizable taxon clearly distinct from the genera *Mycobacterium* and *Nocardia*.

Cross & Goodfellow (1973, Table 16) list a number of named strains which they believe should be allocated to the '*Myco. rhodochrous*' complex. These are in good agreement with my results but I would add *Arth. variabilis* and possibly *Cor. paurometabolum*.

There are at least two main groups in this complex and the heterogeneity of the taxon is in keeping with the results of Bradley (1971), Goodfellow (1971) and Goodfellow *et al.* (1972). The proposal of Tsukamura (1971) that *Myco. rhodochrous* should be allocated to his new genus *Gordona* is premature because he studied only six strains of *Myco. rhodochrous* and none of these was recovered in any of the named species in the genus.

Brevibacterium. As noted by Bousfield (1972) the genus *Brevibacterium* as presently constituted (*Bergey's Manual of Determinative Bacteriology*, 1957) does not appear to be a good taxon. Strains received as *Brevibacterium* spp. are scattered in a number of different clusters in the present study. However, the formation of a distinct cluster by strains of

Table 5. *Characteristics of subgroups F1, F2, F3, F4 and F5 as percentages of positive reactions*

Information derived from material presented in Figs. 3 and 4.

	<i>Kurthia</i> F1	<i>Brevibacterium</i> F4, F5	<i>Myco.</i> <i>rhodochrous</i> F2	<i>Myco.</i> <i>rhodochrous</i> F3
No. of strains tested	7	9	14	20
Colonial morphology				
Colony elevation flat	86	11	0	0
Colony elevation raised	0	11	43	0
Colony elevation convex	14	78	43	90
Colony elevation irregular	0	0	14	10
Colony mucoid	0	0	0	50
Colony leaves impression in agar	100	22	57	90
Colony margin entire	0	100	86	90
Colony margin irregular	100	0	7	10
Colony easily emulsified	100	100	93	85
Colony coarse suspension	0	0	0	15
Colony violet/blue	0	11	0	0
Colony pink/red	0	11	64	65
Colony yellow/orange/brown	86	67	29	20
Colony cream/grey/white	14	11	7	15
Exopigment, water soluble	0	22	64	0
Crystals formed	0	0	0	0
Mycelium formed	0	0	7	55
Aerial hyphae	0	0	7	10
Blue/green iridescence (Henry technique)	0	0	0	0
Stained preparations				
Rods and filaments	71	0	0	5
Rods and cocci	29	89	57	95
Rods only	14	11	29	15
Branching present	0	0	21	45
Mycelium present	0	0	14	15
Cystites	0	0	0	0
Chains formed	100	22	0	10
Barred staining	100	89	86	100
Bipolar staining	0	11	0	0
Rods < 2 μ m long	0	78	71	60
Rods > 2 μ m long	100	22	29	40
Acid fastness	0	11	43	45
Homogeneous growth in SEB	100	100	71	60
Collar/pellicle in SEB	57	56	93	100
Growth on blood agar	100	100	100	100
β -Haemolysis	0	11	0	0
Greening of blood	86	89	21	65
Motility	86	22	29	25
Microcolonies	0	44	71	60
Flagella	71	11	21	20
Growth at				
4 °C	86	100	50	90
10 °C	86	100	86	90
25 °C	100	100	100	100
30 °C	100	100	100	100
37 °C	86	22	29	0
45 °C	57	0	7	0
50 °C	0	0	0	0
Survival at 60 °C for 30 min	0	22	36	30
Anaerobic growth	0	11	71	95
Catalase	100	100	100	100

Table 5 (cont.)

	<i>Kurthia</i> F1	<i>Brevibacterium</i> F4, F5	<i>Myco.</i> <i>rhodochrous</i> F2	<i>Myco.</i> <i>rhodochrous</i> F3
Benzidine	100	100	100	100
Oxidase	0	78	0	5
Acid from				
Arabinose	0	0	0	0
Xylose	0	0	7	0
Rhamnose	0	0	0	0
Fructose	0	33	7	10
Galactose	0	0	0	5
Glucose				
Aerobic	0	11	14	10
Anaerobic	0	11	7	5
Mannose	0	22	14	0
Cellobiose	0	0	0	0
Lactose	0	0	0	0
Sucrose	0	0	7	10
Maltose	0	0	7	5
Melezitose	0	0	0	0
Raffinose	0	0	0	0
Melibiose	0	0	0	0
Sorbitol	0	0	0	0
Salicin	0	0	0	15
Adonitol	0	0	0	0
Inositol	0	11	0	5
Dulcitol	0	0	0	0
Mannitol	0	0	14	0
Glycerol	57	0	7	10
Sorbitol	0	0	7	0
Hydrolysis tests				
Aesculin blackening	0	0	43	90
Aesculin loss purple fluorescence	14	11	29	95
Arginine	100	100	100	100
Arginine (Thornley)	14	0	0	0
Casein	0	33	0	10
Cellulose	0	0	0	0
Chitin	0	0	0	0
Gelatin	0	22	0	10
DNA	100	44	0	55
Hippurate	100	67	36	30
Tween 20 clearing	0	22	0	0
Tween 20 ppt	57	22	100	100
Tween 40 clearing	0	0	0	0
Tween 40 ppt	14	22	93	100
Tween 60 clearing	0	0	0	0
Tween 60 ppt	100	22	100	100
Tween 80 clearing	0	0	0	0
Tween 80 ppt	0	78	100	100
Tyrosine	0	89	21	65
Pigment from tyrosine	0	11	0	0
Xanthine	0	67	0	0
Starch	0	0	0	0
Starch (in 10 % CO ₂)	0	0	0	0
Sole carbon source				
Citrate	0	0	7	80
Gluconate	14	11	29	85
Glucose	14	44	36	50
Hippurate	0	11	0	5
Malate	0	78	7	65

Table 5 (*cont.*)

	<i>Kurthia</i> F1	<i>Brevibacterium</i> F4, F5	<i>Myco.</i> <i>rhodochrous</i> F2	<i>Myco.</i> <i>rhodochrous</i> F3
Sole carbon source (<i>cont.</i>)				
Propionate	0	44	0	0
Pyruvate	14	56	7	35
Succinate	0	11	0	5
Sole nitrogen source				
(NH ₄) ₂ SO ₄	0	67	100	100
KNO ₃	0	67	100	100
KNO ₃ + Casamino acid	71	89	100	100
Growth in presence of				
NaCl				
5 % (w/v)	100	100	100	100
10 % (w/v)	49	100	57	25
Thallos acetate				
0.01 % (w/v)	71	22	43	40
0.02 % (w/v)	43	0	21	30
0.03 % (w/v)	0	0	14	25
Sodium azide				
0.01 % (w/v)	29	33	43	50
0.02 % (w/v)	0	33	29	35
0.05 % (w/v)	0	22	0	15
Potassium thiocyanate				
2.5 % (w/v)	100	100	100	95
3.75 % (w/v)	100	100	93	95
4.5 % (w/v)	57	100	93	95
Tetrazolium				
0.01 % (w/v)	100	56	86	100
Reduction 0.01 % (w/v)	100	56	86	100
0.1 % (w/v)	0	0	64	65
Reduction 0.1 % (w/v)	0	0	14	65
Potassium tellurite				
0.01 % (w/v)	71	89	100	100
Reduction 0.01 % (w/v)	71	89	100	100
0.025 % (w/v)	0	78	100	90
Reduction 0.025 % (w/v)	0	78	100	90
0.05 % (w/v)	0	78	100	90
Reduction 0.05 % (w/v)	0	78	100	90
Lysis by lysozyme	71	89	21	25
Lysis by lysozyme + EDTA	100	100	29	45
Sensitivity to*				
Penicillin (H)	100	100	79	95
Streptomycin (H)	100	100	100	100
Chloramphenicol (H)	100	100	100	100
Aureomycin (H)	100	100	100	100
Terramycin (H)	100	100	100	100
Erythromycin (H)	100	100	100	95
Tetracycline (H)	100	100	100	100
Sulphonamide (H)	0	56	21	65
Ledermycin (H)	100	100	100	100
Celbenin	100	22	7	0
Kanamycin (H)	100	100	100	100
Penbritin (H)	100	100	100	100
Spiramycin (L)	100	11	93	100
Vancomycin (L)	100	100	100	95
Paromomycin (H)	100	11	100	100
Fucidin (L)	100	33	100	80
Neomycin (H)	100	100	100	100

* (H), high potency; (L), low potency.

Table 5 (cont.)

	<i>Kurtzia</i> F1	<i>Brevibacterium</i> F4, F5	<i>Myco.</i> <i>rhodochrous</i> F2	<i>Myco.</i> <i>rhodochrous</i> F3
Sensitivity to (cont.)				
Bacitracin	100	100	100	100
Novobiocin (H)	100	11	100	95
Oleandomycin (H)	100	100	100	100
Colomycin (L)	86	100	100	35
Polymyxin (L)	100	100	100	100
Nitrofurantoin	100	33	100	100
Methyl red	0	0	0	0
Voges Proskauer	0	11	0	0
Egg yolk ppt	0	0	7	0
Egg yolk clearing	0	0	0	0
Litmus milk				
Acid production	0	0	0	0
Alkaline production	100	100	100	100
Coagulation	0	0	0	0
Digestion	0	0	0	0
Peptonization	14	22	0	10
Reduction	57	0	0	10
Reduction NO ₃ → NO ₂	0	33	57	40
Reduction NO ₃ → N ₂	0	0	0	0
Reduction NO ₃ beyond N ₂	0	0	0	0
Production H ₂ S	86	89	100	95
Urease	0	56	79	90
Sulphatase	0	11	0	0
Phosphatase	71	100	100	100
Ammonia from SEB	100	78	86	95

B. linens, the type species, and the association with this group of *B. ammoniogenes* and *B. stationis* lends support to the case made for the retention of the name *Brevibacterium* but with an emended description as suggested by Yamada & Komagata (1972b).

The three strains received as *Corynebacterium* and *Arthrobacter*, which grouped with the two *B. linens* strains to form a tight cluster (F4, see Figs. 2 and 4), all contained a carotenoid-like material which appears to be characteristic of *B. linens* strains (Jones, Watkins & Erikson, 1973). The position of NCIB8179 in this cluster, however, is not easily explained. This bacterium was deposited as *Pseudomonas iodinum*, but was considered by Sneath (1960) to be either a *Corynebacterium* or a *Brevibacterium* and was reclassified by Colwell, Citarella, Ryman & Chapman (1969) as *Arth. iodinum*. All attempts to demonstrate the presence of the *Br. linens* carotenoid-like material failed. It is possible that it is a carotenoid-less mutant but it would additionally have had to develop the purple crystals of iodinin.

Mycobacterium and *Nocardia*. With the exception of *N. cellulans*, *N. calcarrea*, *N. opaca* and *N. turbata*, all the *Nocardia* strains clustered together.

Because so few *Mycobacterium* strains were included, little can be said about the relationship of the two taxa. The grouping of the *Mycobacterium* strains with *Nocardia* spp. (see Figs. 2 and 4) is probably due both to this and to the fact that the tests chosen were more suitable for differentiation in the corynebacteria–arthrobacter area.

Plant pathogenic corynebacteria. The *Corynebacterium* spp. from plant sources did not group as one cluster. All (with the exception of *Cor. insidiosum* which was placed as a satellite strain between areas I and II) clustered in area III.

The results indicate that *Cor. fascians* should be reclassified in the ‘*Myco. rhodochrous*’

Table 6. *Characteristics of Arthrobacter, plant pathogenic corynebacteria and Nocardia as percentages of positive reactions*

Information derived from material presented in Figs. 2 and 4.

	<i>Arthrobacter</i> F6 and F7	<i>Arthrobacter</i> F8	<i>Arthrobacter</i> F9	<i>Arthrobacter</i> F10	<i>Arthrobacter</i> <i>A. oxydans</i>	<i>Arthrobacter</i> F11	<i>Arthrobacter</i> F12	Plant pathogenic bacteria G	<i>Mycobacteria</i> and <i>Nocardia</i> H
No. of strains tested	15	8	13	3	2	8	11	3	16
Colonial morphology									
Colony elevation flat	7	0	0	0	0	0	0	0	0
Colony elevation raised	0	38	15	0	0	63	73	33	13
Colony elevation convex	93	63	85	100	100	38	27	66	38
Colony elevation irregular	0	0	0	0	0	0	0	0	56
Colony mucoid	7	13	0	0	0	0	9	33	6
Colony leaves impression in agar	80	88	77	100	50	50	64	33	100
Colony margin entire	93	100	100	100	100	100	100	100	13
Colony margin irregular	7	0	0	0	0	0	0	0	88
Colony easily emulsified	27	100	100	100	100	100	100	100	44
Colony coarse suspension	0	0	0	0	0	0	0	0	50
Colony violet/blue	0	0	0	66	0	0	0	0	0
Colony pink/red	0	0	0	0	0	0	0	0	6
Colony yellow/orange/brown	47	100	0	0	50	50	100	100	56
Colony cream/grey/white	53	0	100	33	50	50	0	0	38
Exopigment, water soluble	27	13	0	33	50	0	27	0	13
Crystals formed	0	0	0	0	0	0	9	0	0
Mycelium formed	0	0	0	0	0	0	9	0	13
Aerial hyphae	0	0	0	0	0	0	0	0	88
Blue/green iridescence (Henry technique)	0	0	0	0	0	0	0	0	0
Stained preparations									
Rods and filaments	0	0	0	0	0	0	0	0	0
Rods and cocci	80	100	100	100	100	100	82	0	56
Rods only	13	0	0	0	0	0	18	100	0
Branching present	13	13	15	0	0	0	27	0	100
Mycelium present	0	0	0	0	0	0	0	0	94
Cystites	7	0	15	0	0	0	0	0	6
Chains formed	7	0	0	0	0	0	0	0	0
Barred staining	80	100	100	100	100	100	100	66	88
Bipolar staining	0	0	0	0	0	0	0	0	0
Rods < 2 μ m long	87	63	69	100	50	75	100	66	13

Table 6 (cont.)

	Arthrobacter F6 and F7	Arthrobacter F8	Arthrobacter F9	Arthrobacter F10	Arthrobacter <i>A. oxydans</i>	Arthrobacter F11	Arthrobacter F12	Plant pathogenic coryne- bacteria G	<i>Mycobacteria</i> and <i>Nocardia</i> H
Stained preparations (cont.)									
Rods > 2 µm long	13	38	31	0	50	25	0	33	88
Acid fastness	13	63	0	0	50	38	9	0	81
Homogeneous growth in SEB	27	100	100	100	100	100	73	100	0
Collar/pellicle in SEB	40	63	46	66	100	25	82	66	50
Growth on blood agar	93	100	100	100	100	100	100	100	100
β-Haemolysis	0	0	0	0	0	0	0	0	0
Greening of blood	20	100	100	100	100	63	64	0	13
Motility	7	63	38	0	50	75	64	33	0
Microcolonies	40	25	15	0	0	63	9	100	69
Flagella	7	63	38	0	0	50	45	100	0
Growth at									
4 °C	87	100	85	100	100	88	91	100	6
10 °C	100	100	100	100	100	100	100	100	13
25 °C	100	100	100	100	100	100	100	100	81
30 °C	100	100	100	100	100	100	100	100	100
37 °C	7	50	23	66	0	25	9	100	50
45 °C	0	0	0	0	0	0	9	0	19
50 °C	0	0	0	0	0	0	0	0	6
Survival at 60 °C for 30 min	7	0	0	0	0	0	9	0	6
Anaerobic growth	40	0	8	33	0	25	55	0	13
Catalase	100	100	100	100	100	100	100	100	100
Benzidine	100	100	100	100	100	100	100	100	100
Oxidase	20	88	31	66	100	38	36	33	0
Acid from									
Arabinose	20	0	0	0	0	13	36	33	0
Xylose	13	0	0	33	0	13	55	100	0
Rhamnose	33	0	23	33	0	0	45	33	0
Fructose	27	13	92	66	50	13	36	100	13
Galactose	13	0	0	0	0	0	36	100	0
Glucose									
Aerobic	33	13	0	100	0	0	73	100	13
Anaerobic	27	38	0	100	0	13	55	100	13
Mannose	33	0	0	33	0	0	55	100	13
Cellobiose	7	13	0	0	0	0	64	0	6
Lactose	13	0	0	0	0	13	27	0	0

Table 6 (cont.)

Acid from (cont.)	Arthrobacter F6 and F7		Arthrobacter F8		Arthrobacter F9		Arthrobacter F10		Arthrobacter A. oxydans		Arthrobacter F11		Arthrobacter F12		Plant pathogenic bacteria G		Mycobacteria and Nocardia H	
Sucrose	27	13	23	66	100	0	55	66	100	0	55	66	55	66	13	13	13	13
Maltose	33	0	0	0	0	13	36	0	0	13	36	0	36	0	13	13	13	13
Melezitose	0	0	0	0	0	0	27	0	0	0	27	33	27	33	0	0	0	0
Raffinose	0	0	8	0	0	13	18	0	0	13	18	0	18	0	0	0	0	0
Melibiose	20	0	0	0	0	13	18	0	0	13	18	0	18	0	0	0	0	0
Sorbitose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salicin	7	0	0	0	0	0	27	0	0	0	27	100	27	100	6	6	6	6
Adonitol	0	0	0	0	0	0	9	0	0	0	9	66	9	66	0	0	0	0
Inositol	0	0	23	0	0	0	9	0	0	0	9	100	9	100	0	0	0	0
Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mannitol	13	13	31	100	50	0	55	100	50	0	55	0	55	0	0	0	0	0
Glycerol	0	25	0	0	0	13	55	0	0	13	55	66	55	66	0	0	0	0
Sorbitol	7	0	0	33	50	0	0	33	50	0	0	0	0	0	0	0	0	0
Hydrolysis tests																		
Aesculin blackening	73	88	69	100	50	63	100	100	50	63	100	100	100	100	94	94	94	94
Aesculin loss purple fluorescence	60	88	46	100	0	25	100	100	0	25	100	100	100	100	69	69	69	69
Arginine	80	100	100	100	100	100	100	100	100	100	100	100	100	100	81	81	81	81
Arginine (Thornley)	0	0	8	0	0	0	0	0	0	0	0	66	0	66	6	6	6	6
Casein	60	100	92	100	100	75	36	100	100	75	36	100	36	100	31	31	31	31
Cellulose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chitin	7	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6	6	6
Gelatin	33	100	69	66	50	100	73	66	50	100	73	100	73	100	19	19	19	19
DNA	73	88	100	100	100	100	91	100	100	100	91	33	91	33	31	31	31	31
Hippurate	40	100	100	100	100	63	73	100	100	63	73	100	73	100	38	38	38	38
Tween 20 clearing	13	50	46	33	50	38	9	33	50	38	9	0	9	0	6	6	6	6
Tween 20 ppt	93	100	54	66	100	100	82	66	100	100	82	66	82	66	100	100	100	100
Tween 40 clearing	7	13	38	0	0	0	0	0	0	0	0	66	0	66	13	13	13	13
Tween 40 ppt	100	50	69	66	0	100	91	66	0	100	91	100	91	100	94	94	94	94
Tween 60 clearing	7	0	31	0	0	0	0	0	0	0	0	0	0	0	9	9	9	9
Tween 60 ppt	100	0	54	66	50	100	100	66	50	100	100	100	100	100	100	100	100	100
Tween 80 clearing	20	25	85	66	100	63	27	66	100	63	27	0	27	0	13	13	13	13
Tween 80 ppt	93	25	62	33	50	75	73	33	50	75	73	100	73	100	94	94	94	94
Tyrosine	60	100	100	100	100	100	91	100	100	100	91	0	91	0	38	38	38	38
Pigment from tyrosine	47	50	15	0	0	25	9	0	0	25	9	0	9	0	25	25	25	25
Xanthine	0	100	69	0	100	38	0	0	100	38	0	0	0	0	0	0	0	0

Table 6 (cont.)

	Arthrobacter F6 and F7	Arthrobacter F8	Arthrobacter F9	Arthrobacter F10	Arthrobacter <i>A. oxydans</i>	Arthrobacter F11	Arthrobacter F12	Plant pathogenic bacteria G	<i>Mycobacteria</i> and <i>Nocardia</i> H
Hydrolysis tests (cont.)									
Starch	40	63	38	100	100	63	45	33	0
Starch (in 10 % CO ₂)	47	63	38	100	100	63	27	33	0
Sole carbon source									
Citrate	7	0	31	0	0	13	0	0	0
Gluconate	20	100	69	66	100	75	91	100	44
Glucose	53	75	92	100	100	75	91	100	50
Hippurate	0	0	0	0	0	0	0	0	0
Malate	27	13	8	33	0	88	36	0	19
Propionate	0	0	0	0	0	25	0	0	0
Pyruvate	20	13	31	66	50	88	36	0	25
Succinate	7	0	0	0	0	25	0	0	0
Sole nitrogen source									
(NH ₄) ₂ SO ₄	60	75	92	100	100	100	64	66	100
KNO ₃	87	100	92	100	100	100	73	100	100
KNO ₃ + Casamino acid	93	100	92	100	100	100	91	100	100
Growth in presence of									
NaCl									
5 % (w/v)	67	100	77	100	100	100	100	100	63
10 % (w/v)	13	0	0	0	0	75	18	0	19
Thallous acetate									
0.01 % (w/v)	7	13	0	100	100	38	45	33	13
0.02 % (w/v)	7	13	0	100	100	13	36	33	0
0.03 % (w/v)	7	13	0	100	100	13	36	33	0
Sodium azide									
0.01 % (w/v)	13	0	8	33	0	75	0	0	31
0.02 % (w/v)	7	0	0	0	0	38	0	0	0
0.05 % (w/v)	20	0	0	0	50	0	0	0	0
Potassium thiocyanate									
2.5 % (w/v)	53	100	54	100	100	100	100	100	56
3.75 % (w/v)	27	75	23	100	100	100	91	100	56
4.5 % (w/v)	13	63	8	100	100	75	91	100	44
Tetrazolium									
0.01 % (w/v)	53	100	85	100	0	100	100	100	38
Reduction 0.01 % (w/v)	53	100	85	100	0	100	100	100	38
0.1 % (w/v)	0	13	0	0	0	0	45	33	0
Reduction 0.1 % (w/v)	0	13	0	0	0	0	45	33	0

Table 6 (cont.)

Growth in presence of (cont.)	Arthrobacter F6 and F7		Arthrobacter F8		Arthrobacter F9		Arthrobacter F10		Arthrobacter A. oxydans		Arthrobacter F11		F12		Plant pathogenic corynebacteria G	Mycobacteria and Nocardia H
	F6	F7	F8	Arthrobacter	Arthrobacter	Arthrobacter	Arthrobacter	Arthrobacter	Arthrobacter	A. oxydans	Arthrobacter	F11	Arthrobacter	F12		
Potassium tellurite																
0.01 % (w/v)	40		0			31	66		100		100	100	36		66	94
Reduction 0.01 % (w/v)	40		0			31	66		100		100	100	36		66	94
0.025 % (w/v)	20		0			0	0		50		63	63	0		33	75
Reduction 0.025 % (w/v)	20		0			0	0		50		63	63	0		33	75
0.05 % (w/v)	0		0			0	0		0		38	38	0		33	50
Reduction 0.05 % (w/v)	0		0			0	0		0		38	38	0		33	50
Lysis by lysozyme	87		100			92	66		100		75	75	100		100	19
Lysis by lysozyme + EDTA	93		100			100	100		100		75	75	100		100	19
Sensitivity to*																
Penicillin (H)	60		100			100	100		100		100	100	82		100	50
Streptomycin (H)	100		100			100	100		100		100	100	100		100	94
Chloramphenicol (H)	100		100			100	100		100		100	100	100		100	94
Aureomycin (H)	100		100			100	100		100		100	100	100		100	94
Terramycin (H)	100		100			100	100		100		100	100	100		100	94
Erythromycin (H)	93		100			100	100		100		100	100	100		100	94
Tetracycline (H)	100		100			100	100		100		100	100	100		100	100
Sulphonamide (H)	47		75			92	33		100		50	50	45		66	31
Ledermycin (H)	100		100			100	100		100		100	100	100		100	88
Celbenin	33		63			92	100		100		63	63	45		0	31
Kanamycin (H)	100		100			100	100		100		100	100	100		100	100
Penbritin (H)	87		100			100	100		100		100	100	91		100	63
Spiramycin (L)	93		100			100	100		100		100	100	91		100	94
Vancomycin (L)	87		100			100	100		100		100	100	100		100	94
Paromomycin (H)	100		100			100	100		100		100	100	100		100	94
Fucidin (L)	87		100			100	100		100		75	75	100		0	81
Neomycin (H)	100		100			100	100		100		100	100	100		100	88
Bacitracin	100		100			100	100		100		100	100	100		100	94
Novobiocin (H)	93		100			100	100		100		100	100	100		100	81
Oleandomycin (H)	93		100			100	100		100		100	100	100		100	94
Colomycin (L)	80		25			92	100		100		100	100	55		100	63
Polymyxin (L)	100		88			100	100		100		100	100	100		100	94
Nitrofurantoin	67		100			100	100		100		75	75	45		33	88
Methyl red	0		0			0	0		0		0	0	0		0	6

* (H), high potency; (L), low potency.

Table 6 (cont.)

	<i>Arthrobacter</i> F6 and F7	<i>Arthrobacter</i> F8	<i>Arthrobacter</i> F9	<i>Arthrobacter</i> F10	<i>Arthrobacter</i> <i>A. oxydans</i>	<i>Arthrobacter</i> F11	<i>Arthrobacter</i> F12	Plant pathogenic bacteria G	<i>Mycobacteria</i> and <i>Nocardia</i> H
Voges Proskauer	7	13	0	0	0	0	0	66	13
Egg yolk ppt	20	0	0	0	0	25	9	0	19
Egg yolk clearing	3	100	23	33	0	88	18	0	13
Litmus milk									
Acid production	7	0	0	33	0	0	45	66	0
Alkaline production	67	100	100	66	100	100	18	33	88
Coagulation	0	0	0	33	0	0	0	33	0
Digestion	0	0	0	33	0	0	0	33	0
Peptonization	7	100	54	0	100	100	18	33	19
Reduction	13	0	31	66	100	25	45	66	0
Reduction $\text{NO}_3 \rightarrow \text{NO}_2$	33	0	23	0	0	88	27	0	75
Reduction $\text{NO}_3 \rightarrow \text{N}_2$	0	0	0	0	0	0	0	0	0
Reduction NO_3 beyond N_2	0	0	0	0	0	0	0	0	13
Production H_2S	73	100	100	100	100	100	91	100	56
Urease	0	100	100	100	100	63	9	0	81
Sulphatase	0	0	0	0	0	0	18	0	19
Phosphatase	100	100	100	100	100	100	91	100	81
Ammonia from SEB	60	100	100	100	100	88	82	66	75

area. *Corynebacterium ilicis*, *Cor. michigenense* and *Cor. rathayi* should be reclassified as *Arthrobacter* spp. This is in agreement with the observation of Lelliott (1966) that *Cor. fascians* was probably more closely related to *Nocardia* and with his findings that *Cor. fascians* and *Cor. ilicis* were the only urease-positive members of the group. Similarly Lacey (1955) reported branching and acid-fast nocardia-like cells in the *Cor. fascians* and *Cor. michigenense* strains studied by him.

Rosenthal & Cox (1953) showed a serological relationship between *Cor. michigenense* and *Cor. insidiosum*. This relationship was not confirmed in the present study. However, only one strain of *Cor. michigenense* was included and there do seem to be marked serological differences between different strains of this species (Lelliott, 1966).

The close serological similarity between *Cor. poinsettiae* and *Cor. flaccumfaciens* noted by Rosenthal & Cox (1954) and the relationship between both these species and *Cor. betae* suggested by Lelliott (1966) is confirmed in the present study. However, my results indicate that all should be removed from the genus *Corynebacterium*. Yamada & Komagata (1972*b*) have proposed a new genus *Curtobacterium* (type species *Curtobacterium citreum*) to include certain motile brevibacteria, *Cor. flaccumfaciens* and *Cor. poinsettiae*, and my results indicate that *Cor. betae* may also be placed here.

Ungrouped strains. *Brevibacterium leucinophagum* (Kinney & Werkman, 1960), appeared Gram-negative, with traces of Gram-positive staining. However, enzyme studies showed it to be a Gram-negative bacterium probably of the genus *Acinetobacter* (Jones & Weitzman, 1974). This is in keeping with its position in the present study.

Cellulomonas rossica – *Corynebacterium nephridii*. These two strains also gave equivocal Gram reactions. Clark (1951) was of the opinion that *Cel. rossica* was Gram-negative and in a later paper (Clark, 1952) he recommended its exclusion from the genus *Cellulomonas*. There appears to be little work on *Cor. nephridii*. Unpublished work (P. D. J. Weitzman and D. Jones) indicates that the citrate synthases of both these bacteria have the regulatory properties typical of those found in Gram-negative bacteria.

Brevibacterium acetylicum, *Achromobacter liquefaciens*. These two strains group in area III and show about an 85 % similarity to each other. Both give weak but definite Gram-positive staining reactions and both possess citrate synthase with the regulatory properties of Gram-positive bacteria (Jones & Weitzman, 1971). Little can be said about their taxonomic position.

CONCLUSIONS

As pointed out by Sneath (1972) there is a growing opinion that categories above the rank of species and genus are artificial in bacteriology, but nevertheless they are convenient for referring to groups of related bacteria for purposes of presentation.

In a study of this kind, comprising such diverse bacterial genera, it seems sensible to attempt to summarize the main conclusions and as far as possible indicate the relationships of bacteria in the context of the higher taxa (families) as presently listed in *Bergey's Manual of Determinative Bacteriology* (1957).

1. The genera *Erysipelothrix* and *Listeria* and the species *Micro. thermosphactum* should be removed from the Corynebacteriaceae. A strong case can be made for their inclusion in the family Lactobacillaceae. *Microbacterium thermosphactum* is sufficiently distinct to merit genus status. The characters which differentiate the three species are given in Table 7.

2. The family Corynebacteriaceae contains at least five and probably six taxa worthy of genus rank: (i) a group synonymous with *Corynebacterium* (Lehmann & Neumann) containing *Cor. diphtheriae* and related animal bacteria and *Micro. flavum*, but not *Cor. equi*,

Table 7. Characters which differentiate *M. thermosphactum*, *L. monocytogenes* and *E. rhusiopathiae*

Tests	<i>Micro.</i> <i>thermosphactum</i>	<i>List.</i> <i>monocytogenes</i>	<i>E.</i> <i>rhusiopathiae</i>
Growth at 37 °C	—	+	+
Growth at 4 °C	+	+	—
Colonial appearance			
'Fried egg colony'	+	—	—
'Henry technique'	—	+	—
β -Haemolysis	—	+	weak +
Greening on blood	+	—	—
Catalase	+	+	—
Motility	—	+	—
Growth in presence of			
NaCl (5 %)	+	+	—
NaCl (10 %)	+	+	—
Na azide (0.01 %)	—	—	+
K. thiocyanate (2.5 %)	+	+	—
K. thiocyanate (3.75 %)	—	+	—
Reduction of			
K. tellurite (0.01 %)	—	+	+
K. tellurite (0.25 %)	—	+(19/21)*	+
Tetrazolium (0.01 %)	—	+	—
Hydrolysis of			
Tween 40	—	+(17/21)*	—
Tween 60	—	+	—
Sodium hippurate	—	+	+
Phosphatase	+	+	—
Lysis by lysozyme	+	+	—
Sensitivity to			
Kanamycin	+	+	—
Neomycin	+	+	—
Novobiocin	+	+	—
Voges/Proskauer	+	+	—
Acid from			
Adonitol	+	—	—
Arabinose	—	—	+(4/5)*
Cellobiose	+	+	—
Dulcitol	+(4/5)*	—	+
Glycerol	+	+	—
Inositol	+	—(19/21)*	—
Mannitol	+	—	—
Melibiose	+	—	—
Rhamnose	+	+	—(4/5)*
Salicin	+	+	—
Sorbitol	+	—(19/21)*	—
Sucrose	+	+	—
Growth on gluconate	—	+	—

* No. of strains giving reaction, out of total tested.

Cor. haemolyticum, *Cor. pyogenes* or *Cor. murium*; (ii) a group clustering around *Cor. pyogenes*; (iii) *Cellulomonas* (Clark, 1952) but excluding *Cel. fimi* which should be reclassified as *Arth. fimi*; (iv) *Propionibacterium*; (v) a group based on *List. denitrificans* and strains isolated from other sources; (vi) *Microbacterium* (Orla-Jensen, 1919), excluding *Micro. flavum* and *Micro. thermosphactum* but including strains now referred to as *Br. imperiale*.

3. The genus *Arthrobacter* should be removed from the family Corynebacteriaceae as it shows a closer affinity to area III than to area II. It is a large heterogeneous taxon. *Cellulo-*

monas fimi, *Cor. aquaticum*, *Cor. ilicis*, *Cor. michiganense*, *Cor. rathayi*, *Cor. vesiculare* and *Cor. viscosum* should be reclassified as *Arthrobacter* species.

4. *Brevibacterium* should be retained for the species *Br. linens*, *Br. ammoniogenes* and *Br. stationis*. *Kurthia* is a distinct group of bacteria and there is no evidence to support its inclusion with *Brevibacterium* in the family Brevibacteriaceae.

5. Bacteria named '*Mycobacterium rhodochrous*' together with *Arth. variabilis*, *Cor. equi*, *Cor. fascians*, *Jensenia canicruria*, *N. calcaria*, *N. opaca* and possibly *Cor. paurometabolum* form a distinct taxon separate from the genera *Mycobacterium* and *Nocardia*. There is evidence for at least two clear-cut groupings worthy of generic rank.

6. The plant pathogenic corynebacteria should be removed from the genus *Corynebacterium*.

7. *Nocardia cellulans* and *N. turbata* should be removed from the genus *Nocardia*. The genus *Oerskovia* (Prauser *et al.* 1970) has been proposed for this group and our results support separate genus status distinct from the genus *Cellulomonas*. They group in area II and could be placed in the Corynebacteriaceae.

The results of this study indicate that bacteria presently called 'coryneform' can be allocated to at least three different families. The work presented here provides some clarification of the problems and highlights those areas where further study is required. The relationships which emerge are in good accord with other numerical taxonomic studies of smaller samples of the same bacterial genera and with chemotaxonomic studies.

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