Numerical Taxonomy of Some Named Coryneform Bacteria

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(Accepted for publication 16 January 1969)

SUMMARY

Numerical analysis of data for seventy coryneform bacteria bearing the following generic names, *Mycobacterium*, *Nocardia*, *Jensenia*, *Listeria*, *Erysipelothrix*, *Kurthia*, *Brevibacterium*, *Arthrobacter*, *Cellulomonas*, *Microbacterium* and *Corynebacterium*, was done by using 70 coded features and both single and complete linkage clustering programs.

Three major phenons were detected, probably corresponding taxonomically to the present family level in the taxonomic hierarchy. The first included *Listeria*, *Kurthia*, *Erysipelothrix*, certain *Microbacterium* strains and *Corynebacterium* species of animal origin. The second included rapid-growing *Mycobacterium* species, *Nocardia* and *Jensenia*. The third was a more ill-defined group of *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Microbacterium* and certain other species. We suggest that all three phenons belong in the order Eubacteriales under the possible family names of Corynebacteriaceae, Mycobacteriaceae and Arthrobacteriaceae respectively.

INTRODUCTION

Gram-positive bacteria are currently classified in five major groups. Four of these, the micrococci, sporing bacilli, lactic-acid bacteria and the coryneforms, are considered members of the order Eubacteriales, whilst overtly acid-fast coryneforms and the actinomycetes are placed in a separate order, Actinomycetales. The problems inherent in dividing Gram-positive bacteria into taxa at the generic, familial and ordinal level have been remarked by various workers and the reader is referred to Davis & Freer (1960), Gordon (1966) and Jensen (1966) for material relevant to the coryneform problem in particular. Some of the difficulty stems from the fact that taxonomy of bacteria at the 'alpha' level is based on morphology and, as the name implies, coryneforms are irregular in this respect. More subtle problems are also encountered, however, such as gradation in acid-fastness (Harrington, 1966) and the taxonomic interpretation of cell-wall analysis (Cummins, 1962) and nutritional needs (Keddie, Leask & Grainger, 1966). In attempting to classify bacteria, or anything else for that matter, we assume that natural discontinuities or groupings exist in the material studied and can be detected if looked for. As the amount of information upon which groupings may be made increases, the detection of groups becomes more difficult. Numerical taxonomy (Sokal & Sneath, 1963) offers a practical means of reducing this difficulty by impartial mechanical data analysis.

The present study was an attempt to detect similarity groupings in 70 strains of 'coryneform' bacteria, all of which have been maintained for varying periods in this and other laboratories under the names shown in Table 1. Eleven generic names occur in this list and the strains represent isolates from diverse habitats. An investigation of basically similar design was reported by Da Silva & Holt in 1965.

Table 1. *Strains examined*

Study no.	Name on receipt	Donor*	Donor number
107	<i>Brevibacterium superdae</i>	A	CCEB 366
12	<i>B. linens</i>	B	25
13	<i>B. linens</i>	B	26
92	<i>B. insectiphilium</i>	C	146
91	<i>B. sociovivum</i>	C	135
94	<i>B. imperiale</i>	C	—
106	<i>B. imperiale</i>	A	CCEB 497
104	<i>B. ammoniagenes</i>	A	CCEB 364
93	<i>B. ammoniagenes</i>	C	—
14	<i>B. helvolum</i>	B	67
108	<i>B. protophormiae</i>	A	CCEB 282
105	<i>B. fuscum</i>	A	CCEB 277
109	<i>B. vitarumen</i>	A	CCEB 252
15	<i>Mycobacterium hyalinum</i>	B	119
16	<i>M. phlei</i>	B	120
66	<i>M. fortuitum</i>	—	NCTC 8573
69	<i>M. rhodochrous</i>	—	NCTC 8139
70	<i>M. lacticola</i>	C	011
71	<i>M. lacticola</i>	C	012
134	<i>M. ranae</i>	D	—
5	<i>Nocardia salmonicolor</i>	B	121
6	<i>N. opaca</i>	B	133
8	<i>N. rubropertinca</i>	B	134
9	<i>N. coeliaca</i>	B	132
76	<i>N. rubra</i>	C	—
77	<i>N. corallina</i>	C	NOC 19
101	<i>N. salivae</i>	—	—
132	<i>N. asteroides</i>	B	131
135	<i>Nocardia</i> sp.	E	KARLSON
137	<i>Corynebacterium diphtheriae</i>	F	2650
140	<i>C. renale</i>	G	NCTC 7448
173	<i>C. ovis</i>	G	NCTC 3450
179	<i>C. equi</i>	G	NCTC 1621
180	<i>C. ulcerans</i>	G	48
194	<i>C. murium</i>	G	NCTC 949
217	<i>C. murium</i>	J	115
200	<i>C. xerosis</i>	H	—
237	<i>C. michiganense</i>	I	WU 116
34	<i>Erysipelothrix insidiosa</i>	K	V 535
208	<i>E. insidiosa</i>	F	2682
209	<i>Listeria monocytogenes</i>	F	2681
58	<i>Microbacterium thermosphactum</i>	L	SULZBACHER SW 26
10	<i>M. lacticum</i>	B	275
11	<i>M. lacticum</i>	B	101
59	<i>Microbacterium</i> sp.	L	9
60	<i>Microbacterium</i> sp.	L	10
61	<i>Microbacterium</i> sp.	L	11
62	<i>Microbacterium</i> sp.	L	15
63	<i>Microbacterium</i> sp.	L	22
64	<i>Microbacterium</i> sp.	L	27
65	<i>Microbacterium</i> sp.	L	119
28	<i>Kurthia zopfii</i>	B	92
29	<i>K. zopfii</i>	B	93
99	<i>Jensenia canicruria</i>	—	—
17	<i>Arthrobacter terregens</i>	B	6
242	<i>A. terregens</i>	I	NCIB 8909
18	<i>A. tumescens</i>	B	7
19	<i>A. pascens</i>	B	4

Table 1 (cont.)

Study no.	Name on receipt	Donor*	Donor number
20	<i>A. simplex</i>	B	5
240	<i>A. simplex</i>	I	NCIB 8929
241	<i>A. citreus</i>	I	NCIB 8908
244	<i>A. citreus</i>	I	WU 484
21	<i>A. citreus</i>	B	2
22	<i>A. globiformis</i>	B	3
238	<i>A. globiformis</i>	I	WU 477
239	<i>A. globiformis</i>	I	NCIB 8907
24	<i>A. ureafaciens</i>	B	8
23	<i>A. aurescens</i>	B	I
96	<i>Cellulomonas flavigena</i>	—	NCIB 8073
110	<i>C. biazotea</i>	—	NCIB 8077

* Donors. A, O. Lysenko, Czechoslovak Acad. Sci., Prague; B, E. Szabo, Microbiology Culture Collection, University of Queensland; C, J. V. Bhat, Indian Inst. of Science, Bangalore; D, M. R. McLean, CSR, Roseville, N.S.W.; E, R. Harris, Inst. of Dental Research, Sydney; F, E. F. Crowder, Inst. of Medical and Veterinary Science, Adelaide; G, R. W. Cobb, I.C.I. Research Division, Macclesfield; H, J. C. Keast, Veterinary Research Station, Glenfield, N.S.W.; I, C. A. Parker, Inst. of Agriculture of West Australia, Nedlands; J, R. F. Jones, Dept. of Veterinary Pathology, University of Sydney, N.S.W.; K, G. Simmonds, Animal Research Institute, Brisbane; L, L. Brownlie, C.S.I.R.O. Meat Preservation Laboratory, Brisbane.

METHODS

(a) *Strains studied.* These are listed in Table 1. In view of the possibility of misrepresentation we wish to point out that none of the strains bearing internationally recognized culture collection labels was obtained direct from those collections.

(b) *Strain growth and maintenance.* Initial examination of all strains for purity by multiple subculture on solid media was routinely done. All grew aerobically at 28° on the following medium, subsequently referred to as P.Y.E. medium: peptone, 1% (w/v), yeast extract, 0.5% (w/v), NaCl, 0.5% (w/v); for solid media 1.5% (w/v) Oxoid Agar No. 3 was added. During the test period strains were stored at 4° on P.Y.E. slopes. Inocula for tests were grown from these in P.Y.E. broth incubated at 28°. All strains were lyophilized for long-term storage.

(c) *Data collection.* Unless otherwise indicated all characters were determined at 28° on P.Y.E. medium or with P.Y.E. medium as the nutrient base. All other procedures and timings were standardized to enable direct strain-to-strain comparison. In the following brief descriptions the numbers in parentheses indicate the number of features coded from an observed character.

Forty-eight-hour discrete colonies were observed for colony diameter as under 1 mm. or over 2 mm. (2); margin entire or irregular (1); surface smooth or otherwise (1); elevation convex or flat (1); consistency butyrous, friable or solid (3). Cell morphology determined by Gram-stained smears (Jensen's modification; Cruickshank, 1965), as rods present (1); regular rods (1); irregular rods (1); cocci (1); branching (1). Gram reaction (1). Acid-fast cells present (1) using 20% (v/v) sulphuric acid for 3 min. as decolorising agent. Catalase activity (1) by adding '10 vol.' H₂O₂ to growth smeared on a clean slide. Oxidase reaction (1) by Kovacs' (1956) method.

Duplicate plate cultures incubated in the light and dark were observed at 48 hr for pigmentation in the light, present (1); pink (1); yellow/red (1); yellow/green (1). Dark-

incubated plates were exposed to light for 1 hr, re-incubated in darkness for 24 hr and observed for photochromogenesis (1), i.e. appearance or enhancement of colour.

Growth in the absence of oxygen (1); at 37° (1); on Hoyle medium (Cruickshank, 1965) (1); on MacConkey agar (Oxoid CM5 broth plus 1.5% (w/v) agar) (1); on Levine E.M.B. agar (Oxoid CM69) (1); all recorded after 48 hr.

Sensitivity to penicillin, 15 I.U. (1), streptomycin, 10 µg. (1), tetracycline, 10 µg. (1), chloramphenicol, 10 µg. (1), novobiocin, 5 µg. (1), oleandomycin, 5 µg (1), erythromycin, 10 µg. (1) was determined after 48 hr with Oxoid Multodisk no. 11-14C.

Growth at 5° (1) was determined on plates incubated for 28 days.

Oxidation or fermentation of glucose (2) was tested by using Baird-Parker (1963) base with 1% (w/v) glucose, 0.003% (w/v) bromcresol purple and 1.5% (w/v) agar by Mossel's (1962) single tube method. Acetoin production (1) was detected by Barritt's method (Cruickshank, 1965) after 6 days in P.Y.E. broth plus 1% (w/v) glucose. Final pH below 5.0 (1) was recorded at 6 days in the acetoin tests above.

The neutral red test (1) of Cann & Wilcox (1965) was modified to use P.Y.E. agar and read at 6 days. Clearing of 5% (v/v) horse blood agar (1) was read at 48 hr. Citrate utilization (1) using Simmons' method, and aldehyde formation from glycerol (1) using Stern's method, were read after 14 days (Cruickshank, 1965). Hydrolysis of salicylate (1) (Tsukamura, 1966) and cellulose (1) (Skerman, 1967) were observed for 4 weeks. Hydrolysis of tributyrin (1) (Willis, 1960); Tweens 20, 40, 60 and 80 (4) (Sierra, 1957); gelatin (1) (Frazier, 1926); casein (1) (Davis & Park, 1962); starch (1) (Davis & Park, 1962); DNA (1) (Jeffries, Holtman & Guse, 1957, using Difco DNA-ase test agar) were read after 6 days. Hydrolysis of xanthine and tyrosine (2) (Gordon & Smith, 1955) observed after 14 days. Liquefaction of Loeffler serum slopes (1) after 3 weeks.

Aesculin hydrolysis (1), method of Davis (1955) using P.Y.E. base; arbutin hydrolysis (1) as for aesculin, both read at 14 days. Hippurate hydrolysis (1) (Davis, 1955) read at 6 days. Ammonia from arginine (1) (Davis, 1955) read at 6 days. Thornley's (1960) arginine test (1) was modified to use P.Y.E. base and read at 6 days. Nitrate and nitrite reduction (2) were tested separately as recommended by ZoBell (1932) after 6 days. Indole production (1) was tested for in P.Y.E. broth plus 1% (w/v) Oxoid acid-hydrolysed casein after 6 days by using the Ehrlich-Bohme reagent. Urease (1) was detected in a modified Christensen test (Cruickshank, 1965) at 6 days. A modified gluconate test (1) (Cruickshank, 1965) was read at 6 days.

Diffusible brown pigment on P.Y.E. agar plus 0.1% (w/v) sterile catechol and in the tyrosine test referred to above was recorded at 6 and 14 days respectively (2).

Motility (1) was observed in hanging drop preparations of 48 hr P.Y.E. broth cultures.

A total of 70 features were coded.

(d) *Data analysis.* The simple matching coefficient of Sokal & Sneath (1963) was used for similarity assessment. Two clustering programs were tested, one relying on single linkage analysis and the other on complete (or multiple) linkage. The two programs were prepared by Miss E. Szabo of this department (to be published) and run on a GE 225 computer. The complete linkage analysis was stopped at the 60% *S* level after 21 min. on the computer. The single linkage analysis took 7 min. of computer time and ran to completion.

RESULTS

Several strains showed colonial variation during preliminary culture and were separated into discrete cultures. Three examples of this (strains 61A and B, 63A and B, 92A and B) were included in the study, bringing the total strains studied to 73. Our

Fig. 1. Dendrogram derived from single linkage analysis.

results indicate that in the case of strains 61 and 63 the cultures separated were essentially the same but in the case of 92 the two cultures differed considerably and neither appeared to fit the description of *Brevibacterium insectiphilium*. Similar deviations from published descriptions were found in a number of the strains studied, e.g. strains 109 and 106 were both Gram-negative and motile. Most of the characters

Fig. 2. Dendrogram derived from complete linkage analysis.

recorded in this study have either been tested for in a non-comparable manner or not tested for at all in previous reports. Our objective was not identification but taxonomy and we have used the results obtained without prejudice.

Dendrograms based on the computer outputs using single and complete linkage

Fig. 3. Schematic representation of single linkage clustering.

Fig. 4. Schematic representation of complete linkage clustering.

clustering programs are compared in Figs. 1 and 2. Figures 3 and 4 show these in simplified forms. The unsorted similarity matrix for all the strains with the *S* values shown as percentages is given in Fig. 5.

The numbers of clusters (phenons) formed and the numbers of strains clustered by the two methods at descending levels of similarity are shown in Table 2.

The complete linkage analysis to the 60% *S* level resulted in an incomplete sort and in comparison with the single linkage analysis led to differences in cluster (phenon) formation, particularly at lower *S* levels. One strain, *Nocardia* sp. (KARLSON) 135, stood out from the rest of the strains in both analyses. The single linkage analysis indicated three large clusters at c. 80% *S* covering 57 of the 73 strains. Thirteen sub-clusters were indicated in these strains (A-M in Figs. 1 and 3). By complete linkage three large clusters were formed at the 60-65% *S* level (1, 3 and 4 in Figs. 2 and 4), accounting for 46 strains and subdivisible into 11 subclusters (A-C, F-J, K-M in Figs. 2 and 4). Groups 6 and 8 in Figs. 2 and 4 included seven strains each, divisible in each case into two subclusters.

Table 2. *Comparison of strain clustering and phenon formation levels in single and complete linkage analyses*

<i>S</i> (%)	Single linkage		Complete linkage	
	No. of phenons	No. of strains	No. of phenons	No. of strains
95	2	8	2	4
90	8	24	10	23
85	8	35	10	25
80	6	59	18	51
75	2	69	19	60
70	1	73	18	70
65	—	—	15	72
60	—	—	9	72

Similarities between the two cluster analyses were as follows: Nine strains labelled *Microbacterium* sp. clustered with one named *Microbacterium thermosphactum* (McLean & Sulzbacher, 1953) and the single strain of *Listeria monocytogenes* (Cluster 1A in both analyses). Two strains of *Microbacterium lacticum* were well separated from this cluster and in both analyses they fell into the same major group as a strain of *Corynebacterium michiganense* and one of *Cellulomonas biazotea*. Two strains of *Erysipelothrix* clustered with one labelled *Corynebacterium murium* (194). They eventually linked with the *Microbacterium/Listeria* and the *Kurthia* subclusters in both analyses. A second strain of *Corynebacterium murium* (217) clustered with three other corynebacteria of animal origin (*xerosis*, *ovis*, *diphtheriae*) in both analyses.

Cluster 3 in both analyses represented a clear group of *Mycobacterium* and *Nocardia* species although the exact composition and subclustering varied. *Jensenia canicruria* was included in this group.

Three strains of *Arthrobacter citreus* showed close similarity as also did three strains of *A. globiformis*; *A. pascens* (19), *A. ureafaciens* (23) and *A. aurescens* (24) were linked to the latter strains.

One strain of *Arthrobacter simplex* (20) showed relationship to *Corynebacterium renale* and *C. ulcerans*, which clustered together. *Corynebacterium equi* (179) was well separated from other corynebacteria.

[illegible]

Cellulomonas flavigena (96) associated with a strain of *Brevibacterium ammoniagenes* (93); *Cellulomonas biazotea* (110) was well separated. A second strain of *B. ammoniagenes* (104) linked with a strain of *B. imperiale* (94) which in turn was well separated from the second *B. imperiale* strain (106). The two strains of *B. linens* clustered together. *Brevibacterium fuscum* (105) and *B. protophormiae* (108) were linked and clustered with the *A. citreus* and *A. globiformis* strains; *B. saperdae* (107) and *Mycobacterium hyalinum* (15) fell into the same cluster.

Differences noticeable in the two cluster analyses were the separation of the animal corynebacteria from cluster 1 in the multiple linkage analysis; the breakdown and redistribution of single linkage cluster 2 in the multiple linkage analysis and a similar redistribution of the poorly clustered strains in the lower part of Fig. 1 by the more searching analysis.

DISCUSSION

Apart from the absence of 'sugar fermentation' tests the characters used in this study resemble in general terms those commonly used in recently published papers on bacterial taxonomy by numerical analysis, e.g. Goodfellow (1967); Splittstoesser, Wexler, White & Colwell (1967). Photochromogenesis is a characteristic used in Runyon's diagnostic scheme for anonymous mycobacteria (Runyon, 1955), and depends upon adaptive carotenoid production. It is a factor which needs to be considered in assessing cultures for pigment formation in taxonomic work. The following strains showed this character, *Nocardia rubropertincta* and *Nocardia* sp. (KARLSON), *Mycobacterium hyalinum* and *M. rhodochrous*, *Brevibacterium aureescens*, *B. urea-faciens*, *B. insectiphilum* (92A) and *B. saperdae*. Except for *Nocardia* sp. (KARLSON) these strains clustered in two separate positions, some in the *Nocardia*/*Mycobacterium* cluster and some (including *M. hyalinum*) in the *Arthrobacter globiformis*/*citreus* cluster. Photo-induced carotenogenesis was discussed by Burchard & Dworkin (1966) and Mathews (1966).

The *Mycobacterium*/*Nocardia* cluster of strains is well defined in our results and there is little evidence of linkage between this group and any of the corynebacteria examined. This reinforces Gordon's (1966) conclusion that the genus *Corynebacterium* is not a suitable repository for her *Mycobacterium rhodochrous*. Although there is good evidence against the present division of *Corynebacterium*, *Mycobacterium* and *Nocardia* at the ordinal level we cannot support Harrington's (1966) plea for a common genus. Recent studies on *Mycobacterium* taxonomy by Tsukamura (1966) and Bogdanescu & Racotta (1967) did not cover the problems raised by Gordon's 1966 work although Tsukamura's separation of the rapidly growing and slowly growing species into two groups suggests that it may be possible to recognize a separate taxon composed of rapidly growing mycobacteria and related forms. This would include Gordon's *Mycobacterium rhodochrous* group and resemble the *Mycobacterium*/*Nocardia* group detected in the present study. In view of the fact that the type species of *Nocardia*, *N. asteroides*, would probably be included it seems reasonable to suggest that the generic name *Nocardia* be retained for this taxon in spite of Gordon's desire to restrict it to strains capable of aerial mycelium formation. Such a taxon would clearly be heterogeneous in certain characters, particularly morphology, but others (e.g. DNA base ratios, Hill, 1966, and lipid analysis, Lanéele, Asselineau & Castelnouvo, 1965) would probably uphold it, particularly if strains of *Streptomyces*

currently masquerading as nocardias were eliminated. *Corynebacterium equi* (NCTC 1621), which was included by Gordon in an atypical *M. rhodochrous* group and clustered with mycobacteria in Harrington's work, was not well clustered in the present study. Our strains of *Nocardia opaca*, *N. salivae* (Davis & Freer, 1960, renamed *Rothia dentocariosus* by Georg & Brown, 1967) and *N. coeliaca* were also imperfectly clustered in one or other of the two programs used. Current evidence for the grouping of *Corynebacterium* species into sub-generic taxa is conflicting. Apart from the probable exclusion of *C. haemolyticum* and *C. pyogenes* (Cummins & Harris, 1956) and a possible division into animal and plant corynebacteria (Da Silva & Holt, 1965) opinions differ on what should be done with the present system (see Harrington, 1966, and Lazar, 1968). Our results support the recognition of a taxon for the animal corynebacteria (Fig. 2, Group 6) and although only one plant corynebacterium (*C. michiganense*) was used the resulting clusters support Da Silva & Holt's grouping of this bacterium with strains labelled *Microbacterium lacticum* (Fig. 2, Group 2; Fig. 1, Group 2) and their separation from the *C. diphtheriae* group.

Recent studies of *Microbacterium* taxonomy by Robinson (1966*a, b*) did not include strains of the organism named *Microbacterium thermosphactum* by McLean & Sulzbacher (1953). Robinson recognized the three species *M. flavum*, *M. lacticum* and *M. liquefaciens* but recommended that the first of these be reallocated to the genus *Corynebacterium* on cell-wall and other evidence. He also noted close relationship between *M. lacticum* and *M. liquefaciens*. In our work two strains of *M. lacticum* associated in both cluster analyses with strains labelled *Corynebacterium michiganense*, *Arthrobacter terregens* and *Cellulomonas biazotea*. In Fig. 1 these bacteria fall into the large cluster including *Arthrobacter globiformis* and *A. citreus*. Keddie *et al.* (1966) could not classify a strain of *M. lacticum* with *Arthrobacter*, *Corynebacterium* or *Cellulomonas* on the basis of cell-wall composition but examination of their and other results suggests that the species shows cell-wall similarities with *Arthrobacter terregens* and *A. flavescens*, and inclusion in the *Arthrobacter* group in the present study is not unreasonable.

As pointed out by Barlow & Kitchell (1966), the systematic position of *Microbacterium thermosphactum* is uneasy. It is more similar in certain respects to the lactobacilli than the coryneform microbacteria. The nine strains labelled '*Microbacterium* sp.' in this work were obtained in the study reported by Weidemann (1965) from beef stored in nitrogen at 0°. They clustered well with a strain of *M. thermosphactum* and one of *Listeria monocytogenes* (Group 1A, Figs. 1 and 2). Relationship with certain animal corynebacteria, *Kurthia* and *Erysipelothrix* is also indicated. This group (1 in Figs. 1 and 2) differs from the other large clusters in Fig. 1 and all except Group 6 in Fig. 2 in being largely composed of non-pigmented, regular rods, showing anaerobic glucose metabolism, no reduction of nitrate and with no acid-fast members. The inclusion in this group of representative strains of four generic type species suggests that the subclusters discernible within Group 1 in Figs. 1 and 2 correspond to current genera. With the possible exception of the animal corynebacteria, most of these bacteria would probably be better accommodated with the lactic-acid bacteria than the coryneforms.

Brevibacterium is acknowledged to be a taxon of convenience erected by Breed in 1953. Its heterogeneity is confirmed by the distribution of strains so named in Figs. 1 and 2. Da Silva & Holt (1965) recommend that the type species *B. linens* be reallocated

as *Arthrobacter linens*. Such a move would invalidate the present genus. In our results *B. linens* clustered closely with *Arthrobacter globiformis* upon single linkage analysis but was separated from the *A. citreus/globiformis* group by complete linkage analysis. In the latter analysis three possibly related groups of *Brevibacterium* strains occur, in Groups 4, 5 and 8. In Group 4, five *Brevibacterium* strains associate with the *A. citreus/globiformis* cluster. In Group 8, four strains cluster with *A. tumescens*. No association with the arthrobacters is seen in Group 5. These results suggest, however, that it may be possible to re-allocate most of the strains currently named *Brevibacterium* in generic taxa emerging from the *Arthrobacter* complex. Goodfellow (1967) found that his own results agreed with previous evidence for the separation of *A. simplex* and *A. tumescens* from a group of arthrobacters associated with *A. globiformis*. This subdivision is supported by our results, *A. simplex* and *tumescens* being separated in

Table 3. Percentage incidence of potentially diagnostic characters in the three major groups discernible by single linkage clustering (57 strains)

Character	<i>C. diph- theriae/ Listeria</i> group (20 strains)	<i>Arthro- bacter/ Brevi- bacterium</i> group (24 strains)	<i>Myco- bacterium/ Nocardia</i> group (13 strains)
Irregular rods	20	90	100
Colony convex	40	96	80
Margin entire	20	100	33
Acid-fast	0	0	72
Pigmented	0	75	88
Catechol-induced pigment	5	67	0
Growth anaerobically	90	42	16
Growth at 5°	45	90	8
Growth on MacConkey	75	96	64
Sensitivity to penicillin	95	90	40
Glucose oxidized	15	75	88
Glucose fermented	65	20	0
Aesculin hydrolysed	60	80	24
Starch hydrolysed	20	80	0
Tyrosine hydrolysed	0	80	32
Casein hydrolysed	5	84	0
Gelatin hydrolysed	5	84	8
DNA hydrolysed	5	71	0
Tributylin hydrolysed	60	42	100
Tween 20 hydrolysed	30	62	88
Tween 40 hydrolysed	15	67	100
Tween 60 hydrolysed	15	62	100
Tween 80 hydrolysed	15	25	100
Nitrate reduced	10	54	100
Motile	20	33	0

both analyses from the main group of arthrobacters (cf. Cummins & Harris, 1959). If, as seems likely, the genus *Brevibacterium* is dispersed in reclassification of the arthrobacters, the family Brevibacteriaceae will lose its type genus and its validity in nomenclature. Our results indicate that *Kurthia zopfii* may represent a valid taxon related to the *Listeria/Erysipelothrix/Microbacterium thermosphactum* complex, with probable relationship also to animal corynebacteria. The displacement of *Kurthia*

from the present family Brevibacteriaceae and inclusion at family level (Corynebacteriaceae?) with the above genera would not be unreasonable.

In conclusion we suggest that the present exploratory study indicates the existence of three major phenons in the strains studied. These are:

(a) Animal corynebacteria and other bacteria showing metabolic and other characters of possible relationship to the family Lactobacillaceae (Group 1, Fig. 1; Groups 1 and 6, Fig. 2).

(b) Rapid-growing mycobacteria and *Nocardia* sp.; Family Mycobacteriaceae Order Eubacteriales. (Group 3 in Figs. 1 and 2.)

(c) *Arthrobacter* complex, requiring new family affiliation (Arthrobacteriaceae?) (Groups 2 and 4 (?), Fig. 1; Groups 2, 4, 5, 7, 8 and 9, Fig. 2.)

The two Tables 3 and 4 have been included in an attempt to show the over-all characters of these three phenons based only on features that showed uneven distribution among the three groups.

Table 4. *Percentage incidence of potentially diagnostic characters in the three major groups discernible by complete linkage clustering (70 strains)*

Character	<i>C. diphtheriae</i> / <i>Listeria</i> group (23 strains)	<i>Arthro-</i> <i>bacter</i> / <i>Brevi-</i> <i>bacteria</i> group (32 strains)	<i>Myco-</i> <i>bacterium</i> / <i>Nocardia</i> group (15 strains)
Regular rods	64	27	33
Irregular rods	17	72	92
Colonies convex	39	84	80
Acid-fast	0	0	60
Pigmented	4	68	86
Growth anaerobically	90	59	13
Growth at 37°	51	84	86
Growth at 5°	59	77	26
Growth on MacConkey	77	96	53
Growth on EMB	0	53	26
Sensitive to penicillin	94	71	33
Growth on Hoyle	30	22	80
Glucose oxidized	21	65	86
Glucose fermented	64	27	0
Acetoin produced	69	34	0
Aesculin hydrolysed	51	71	24
Starch hydrolysed	17	62	0
Tyrosine hydrolysed	0	71	33
Casein hydrolysed	17	71	0
Gelatin hydrolysed	17	84	0
DNA hydrolysed	4	62	0
Tributyrin hydrolysed	60	46	92
Tween 20 hydrolysed	43	56	86
Tween 40 hydrolysed	17	71	100
Tween 60 hydrolysed	17	62	100
Tween 80 hydrolysed	21	27	92
Oxidase	9	62	7
Nitrate reduced	13	62	86
Motile	17	28	13

The authors thank Miss A. Buckridge for technical assistance and all those who donated strains used in this study.

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APPENDIX

Table A shows the coded features and the numbers used to indicate them. Table B lists the results for these features for all the strains used.

Table A

Feature no. in Table B	Coded feature	Feature no. in Table B	Coded feature
1	Colony under 1 mm. in diameter	36	Chloramphenicol sensitive
2	Colony over 2 mm. in diameter	37	Novobiocin sensitive
3	Margin entire	38	Oleandomycin sensitive
4	Surface smooth	39	Erythromycin sensitive
5	Consistency butyrous	40	Growth on Hoyle's medium
6	Colony adherent	41	Growth anaerobically
7	Colony friable	42	Growth at 5°
8	Colony convex	43	Growth at 37°
9	Colony pink	44	Cellulose hydrolysed
10	Colony yellow/green	45	Aesculin hydrolysed
11	Colony yellow/red	46	Arbutin hydrolysed
12	Regular rod forms seen	47	Glucose oxidised
13	Irregular rod forms seen	48	Glucose fermented
14	Branching rod forms seen	49	Acetoin from glucose
15	Cocci seen	50	Stern's test positive
16	Motile	51	Hippurate hydrolysed
17	Acid-fast cells present	52	Nitrate reduced
18	Catalase positive	53	Nitrite reduced
19	Oxidase positive	54	Indole produced
20	Tween 20 hydrolysed	55	Urea hydrolysed
21	Tween 40 hydrolysed	56	Ammonia from arginine
22	Tween 60 hydrolysed	57	Thornley's test positive
23	Tween 80 hydrolysed	58	Citrate utilized
24	Gelatin hydrolysed	59	Gram positive
25	Casein hydrolysed	60	Photochromogenic
26	Starch hydrolysed	61	Tributyrin hydrolysed
27	Tyrosine hydrolysed	62	Horse blood cleared
28	Melanin from tyrosine	63	Neutral red test
29	Xanthine hydrolysed	64	Gluconate test
30	Salicylate hydrolysed	65	Pigment on catechol
31	Growth on MacConkey	66	Serum hydrolysed
32	Growth on EMB	67	DNA hydrolysed
33	Penicillin sensitive	68	Pigment produced
34	Streptomycin sensitive	69	Rod forms seen
35	Tetracycline sensitive	70	Final pH under 5.0

Table B (cont.)

[illegible]

Table B (cont.)

[illegible]

Table B (cont.)

	No.	Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
	92 ^B	<i>B. insectiphilum</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	93	<i>B. ammoniagenes</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	94	<i>B. imperiale</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	104	<i>B. ammoniagenes</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	105	<i>B. fuscum</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	106	<i>B. imperiale</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	107	<i>B. saperae</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	108	<i>B. protophormiae</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	109	<i>B. vitarumen</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	96	<i>Cellulomonas flavigena</i>	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	110	<i>C. biazotea</i>	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	No.	Name	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
	92 ^B	<i>B. insectiphilum</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	93	<i>B. ammoniagenes</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	94	<i>B. imperiale</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	104	<i>B. ammoniagenes</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	105	<i>B. fuscum</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	106	<i>B. imperiale</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	107	<i>B. saperae</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	108	<i>B. protophormiae</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	109	<i>B. vitarumen</i>	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	96	<i>Cellulomonas flavigena</i>	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	110	<i>C. biazotea</i>	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+