Journal of General Microbiology (1972), 71, 441–455
Printed in Great Britain

A Taxonomic Study of Some Coryneform Bacteria

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(*Accepted for publication* 14 *February* 1972)

SUMMARY

Numerical analysis has been carried out on 110 features of 158 named and unnamed coryneform bacteria. At the 30% S-level, four phena of unequal size were formed, the largest of which (phenon II) was divided into two subphena at the 45% S-level representing the genera *Arthrobacter* and *Nocardia*. Phenon III was divided into two subphena at the 45% S-level (IIIA and IIIB). Subphenon IIIA was made up largely of Gram-positive strains received as *Flavobacterium*. Subphenon IIIB contained a variety of strains including a group of cellulomonas-like organisms. Phenon IV was divided into two subphena at the 35% S-level representing the animal-pathogenic corynebacteria and *Microbacterium flavum* respectively. Phenon V contained six strains of which five were plant pathogenic corynebacteria. DNA base composition determinations were carried out on representative strains and the values obtained generally correlated well with the numerical groupings. Considerable reorganization of most coryneform genera was considered necessary and suggestions for the reclassification of species and of particular strains have been made.

INTRODUCTION

The taxonomic difficulties associated with the group of coryneform bacteria have been reviewed by Clark (1952), Jensen (1952, 1953, 1966), Gibson (1953), Bousfield (1969) and Veldkamp (1970). The coryneform group, which is characterized mainly on a morphological basis, is considered to include the genera *Corynebacterium*, *Arthrobacter*, *Brevibacterium*, *Microbacterium*, *Cellulomonas*, *Listeria*, *Erysipelothrix*, *Mycobacterium* and certain species of *Nocardia*, but the equivocal definition of most of these genera causes difficulties in identification.

In recent years, studies of the taxonomy of coryneform bacteria by numerical methods have included those of Da Silva & Holt (1965), Chatelain & Second (1966), Harrington (1966), Mullakhanbhai & Bhat (1967), Splitstoesser, Wexler, White & Colwell (1967), Davis, Fomin, Wilson & Newton (1969), Davis & Newton (1969), Masuo & Nakagawa (1969) and Skyring & Quadling (1970). Non-numerical studies have included those of Keddie, Leask & Grainger (1966), Robinson (1966*a*, *b*, *c*, *d*), Abe, Takayama & Kinoshita (1967), Komagata, Yamada & Ogawa (1969) and Yamada & Komagata (1970*a*, *b*). At the time that the present work was carried out only the reports of Bouisset, Breuillard & Michel (1963) and Abe *et al.* (1967) were available on the DNA base composition of coryneform bacteria. Recently, Skyring & Quadling (1970) and Yamada & Komagata (1968, 1970*b*) have added further information. The present account combines a numerical analysis of a range of properties of coryneform bacteria with the determination of the DNA base composition of representative strains.

METHODS

Strains examined. A hundred and fifty-eight strains were subjected to numerical analysis, including representatives of the genera *Corynebacterium*, *Arthrobacter*, *Brevibacterium*, *Mycobacterium*, *Microbacterium*, *Cellulomonas*, *Nocardia*, *Listeria* and *Erysipelothrix*. In addition, several Gram-positive and Gram-variable organisms deposited with the National Collection of Marine Bacteria as species of *Flavobacterium* were included. Thirty-six new isolates from sea water, fish and soil were also included. Twelve original isolates from soil, cheese and activated sludge and four from surfaces of plants were kindly supplied by Professor E. G. Mulder of Wageningen, Holland, and by Dr A. M. Paton of the University of Aberdeen respectively. The strains used in this study are listed in Table 1. Those obtained from culture collections have the names and catalogue numbers they bore when received. Those from other sources have the code numbers they carried at the time of receipt. New isolates are indicated by code numbers assigned to them by the author at the time of isolation.

Maintenance of strains. All strains were stored as freeze-dried cultures. Active cultures were maintained on nutrient agar (Oxoid CM55, Oxoid Ltd, London, S.E. 1) with the exceptions of the animal and plant pathogens, which were maintained on blood agar slopes and slopes of nutrient agar containing 1% (w/v) glucose respectively.

Numerical analysis. Unless otherwise stated the Oxoid nutrient media CM55 (solid) and CM67 (liquid) were used as the nutrient bases for all tests. Horse serum, 5% (v/v) (Burroughs Wellcome, Beckenham, Kent), was added for the animal pathogens. The latter organisms were incubated at 37 °C for 7 days and all other strains were incubated at 25 °C for 14 days unless indicated otherwise.

Voges–Proskauer and methyl red tests, H_2S production from peptone (paper-strip method), acid-fast stain (Ziehl–Neelsen method using 3% (v/v) HCl in ethanol), indole production (1%, w/v, tryptone water; detection by Ehrlich's reagent), liquefaction of gelatin, hydrolysis of starch (plate method), reduction of nitrate, catalase reaction and reactions in litmus milk were tested according to the *Manual of Microbiological Methods* (Society of American Bacteriologists, 1957). Skim milk 1% (w/v) (Difco Laboratories U.K. Division, East Molesey, Surrey), was incorporated into plates of basal medium to test for casein digestion and plates were examined under u.v. light for the production of diffusible, fluorescent pigments. The oxidase reaction was carried out by the method of Kovacs (1956). Heat resistance was tested by the method of Abd-el-Malek & Gibson (1952). Salt tolerance was tested by incubating strains on plates of basal medium to which 2·5%, 5%, 7·5% and 10% (w/v) NaCl respectively had been added. Growth on plates at 0·6 °C and 10 °C was recorded after 3 weeks of incubation and growth at 20 °C, 25 °C, 30 °C and 37 °C after 5 days of incubation.

High-potency 'Sentest' discs (Evans Medical Ltd, Speke, Liverpool) were used to test penicillin sensitivity. Discs were placed on plates seeded with 1 ml of 24 h broth culture and zones of inhibition were looked for daily up to 1 week. Phosphatase activity was tested by the addition of 0·5% (w/v) phenolphthalein diphosphate to plates of basal medium, which were then exposed after incubation to ammonia fumes. Cellulose digestion was tested by the addition of a filter-paper strip to the (liquid) basal medium. Incubation was continued up to 6 weeks. The anaerobic utilization of glucose ('fermentation') was tested by incubating under hydrogen in liquid medium containing 1% (w/v) glucose.

Acid production was tested in 1% (w/v) peptone water containing 1% (v/v) Andrade's indicator and 1% (w/v) of the following carbohydrates or sugar alcohols: adonitol, arabinose, cellobiose, dextrin, dulcitol, fructose, galactose, glucose, glycerol, inulin, inositol, maltose, mannitol, mannose, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, sucrose,

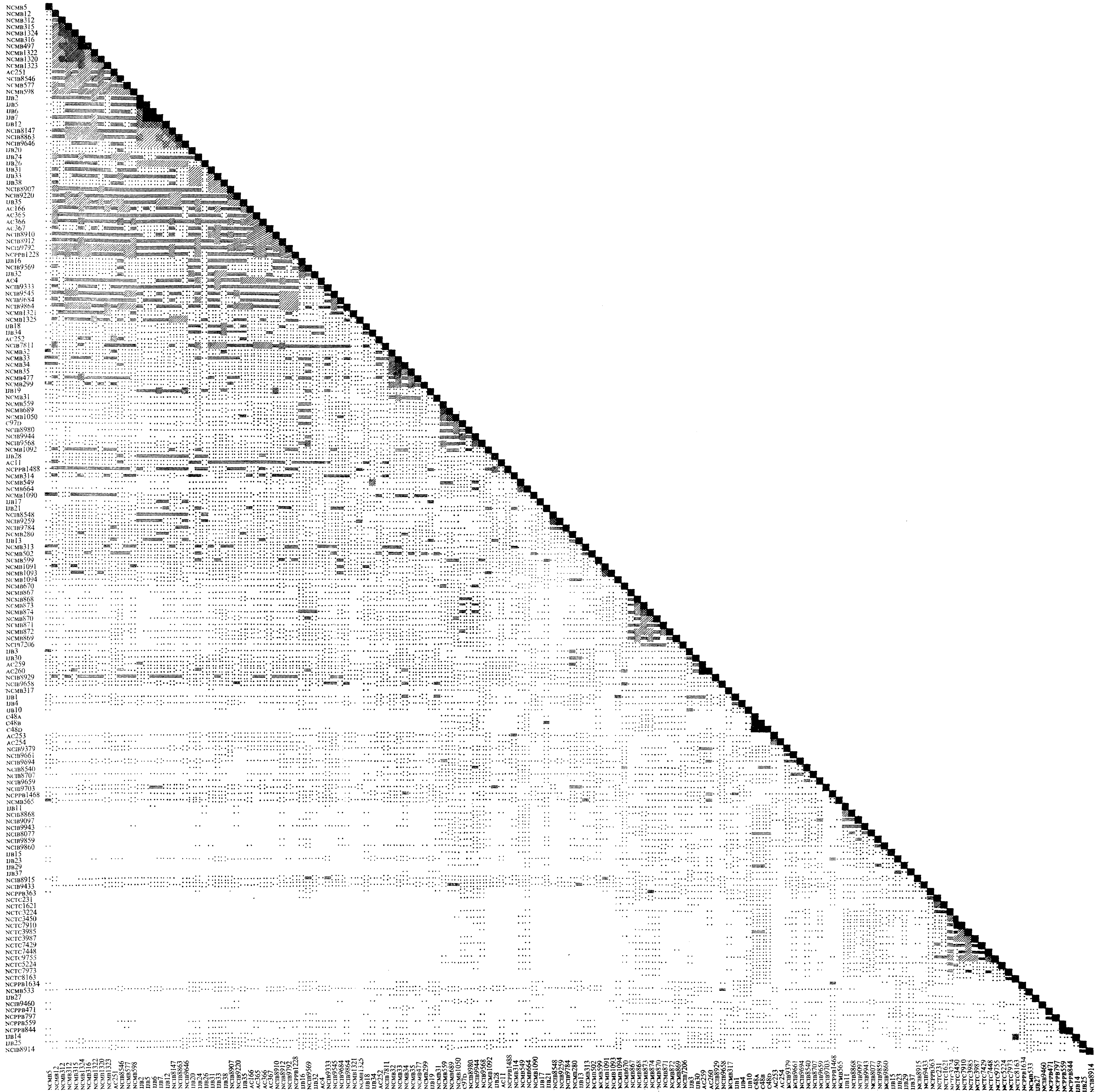

Fig. 1. Diagrammatic representation of complete S-matrix. Key to symbols (% S): ■, 90–100; ▩, 80–89; ▨, 70–79; ═, 60–69; ⁚⁚, 50–59; ••, 40–49.

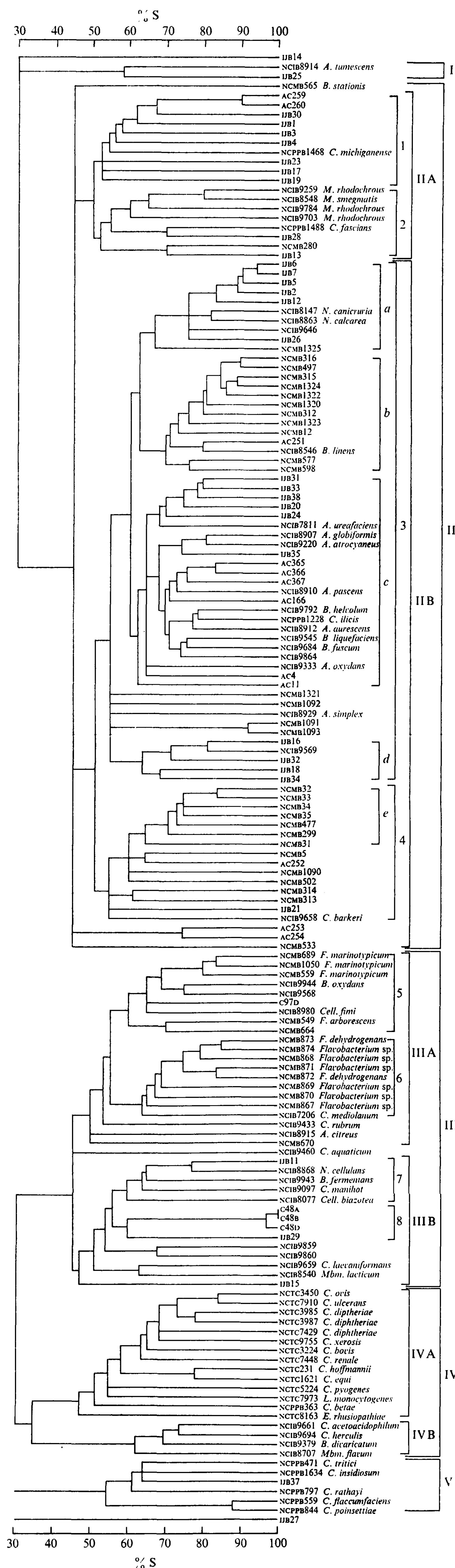

Fig. 2. Dendrogram derived from the S-matrix.

trehalose and xylose. Hiss's serum water sugars (Wilson & Miles, 1964) were used for the animal pathogens.

The utilization of various compounds as sole carbon sources was tested by the replica-plating method of Stanier, Palleroni & Doudoroff (1966). The replicating device was designed and built by the Torry Research Station workshops and is fully described elsewhere (Bousfield, 1969). The basal medium used for the carbon utilization tests was that described by Stanier *et al.* (1966), to which was added (per l) 2·5 ml of the following growth factor solution (L. B. Perry, personal communication): *p*-aminobenzoic acid, 2 mg; folic acid, 2 mg; biotin, 0·4 mg; nicotinic acid, 40 mg; pantothenic acid, 40 mg; thiamin, 40 mg; B_{12}, 0·2 mg; distilled water, 100 ml. Test compounds were incorporated into the basal medium at a concentration of 0·1 % (w/v) and were sterilized by filtration wherever possible, otherwise sterilization was by tyndallization. Compounds tested as sole carbon sources were the sodium salts of acetic, citric, DL-lactic, hippuric, formic, oxalic, succinic, D-malic, fumaric, malonic, *meso*-tartaric, pyruvic, D-mandelic and benzoic acids, phenol, glycine, L-alanine, L-serine, L-threonine, L-leucine, L-valine, L-glutamic acid, L-lysine, DL-arginine, DL-ornithine, γ-aminobutyric acid, L-histidine, L-proline, L-tyrosine, L-phenyl-alanine, cystine, cysteine and methionine. Plates of the basal medium described above with the addition of 1 % (w/v) glucose were used to test strains for the ability to utilize inorganic nitrogen.

Computation of results. Computation of results was carried out by Mr J. C. Gower and Mr H. R. Simpson of the Rothamsted Experimental Station, Harpenden, Hertfordshire, using Gower's classification programme CLASP on the Orion computer. Details of this programme were kindly supplied by Mr H. R. Simpson. Negative matches were not included in the calculation of similarity. Table 2 gives the single features derived from the tests described above. In Table 2, tests marked A are 'alternatives', i.e. multistate tests in which any one of several mutually exclusive features may be recorded. Tests marked Q are 'quantitatives', i.e. any point in a graduated linear series may be recorded. Tests marked D are 'dichotomies' in which the result of a particular test is either positive or negative. For any particular test, a valid comparison between two strains is made only when at least one strain has given a positive result.

Determination of DNA base composition. The isolation and purification of DNA was carried out according to the method of Marmur (1961). The guanine/cytosine (% GC) content was determined by the thermal denaturation method of Marmur & Doty (1962) using the saline/citrate buffer of Marmur (1961). All determinations were carried out using a Unicam SP 800 spectrophotometer (Pye/Unicam Ltd, Cambridge) fitted with a scale expansion unit and an external recorder (Bryan 'Autoplotter', Bryans Ltd, Mitcham, Surrey). Temperature was monitored by means of a plastic coated copper/constantan thermocouple inserted in the sample cuvette.

RESULTS

The complete S-matrix obtained from the numerical analysis is represented diagrammatically in Fig. 1. The dendrogram derived from the S-matrix is shown in Fig. 2 and gives linkages to the 30 % S-level. It can be seen from the dendrogram that four phena of unequal size (phena I to IV) are formed at the 30 % S-level and that one further phenon (V) is formed below the 30 % S-level. Phenon I contains only two strains and will not be discussed further. Phenon II divides at the 45 % S-level into two subphena (IIA and IIB) and three strains. Phenon III divides at the 45 % S-level into two subphena (IIIA and IIIB). Phenon IV divides at the 35 % S-level into two subphena (IVA and IVB). Groups within subphena

Table 1. *Strains used and their sources*

NCIB = National Collection of Industrial Bacteria; NCMB = National Collection of Marine Bacteria; NCTC = National Collection of Type Cultures; NCPPB = National Collection of Plant Pathogenic Bacteria; AC = cultures from E. G. Mulder; C = cultures from A. M. Paton; IJB = strains isolated by the author.

Organism	Strain	Source
Corynebacterium		
acetoacidophilum	NCIB9661	—
aquaticum	NCIB9460*	Distilled water
barkeri	NCIB9658*	Sewage
betae	NCPPB363	Plant
bovis	NCTC3224	Animal
diphtheriae (*gravis*)	NCTC3985	Animal
diphtheriae (*intermedius*)	NCTC3987	Animal
diphtheriae (PW8)	NCTC7429	Animal
equi	NCTC1621*	Animal
erythrogenes	NCMB5	Fish
fascians	NCPPB1488	Plant
flaccumfaciens	NCPPB559	Plant
herculis	NCIB9694*	Soil
hoffmannii	NCTC231	Animal
ilicis	NCPPB1228*	Plant
insidiosum	NCPPB1634	Plant
laevaniformans	NCIB9659*	Activated sludge
manihot	NCIB9097*	Cassava fermentation
mediolanum	NCIB7206	—
michiganense	NCPPB1468	Plant
ovis	NCTC3450	Animal
poinsettiae	NCPPB844	Plant
pyogenes	NCTC5224	Animal
rathayi	NCPPB797	Plant
renale	NCTC7448*	Animal
rubrum	NCIB9433*	Air
tritici	NCPPB471	Plant
ulcerans	NCTC7910	Animal
xerosis	NCTC9755	Animal
Arthrobacter		
atrocyaneus	NCIB9220*	Air
aurescens	NCIB8912	Soil
citreus	NCIB8915	Soil
globiformis	NCIB8907*	Soil
oxydans	NCIB9333	Soil
pascens	NCIB8910*	Soil
simplex	NCIB8929*	Soil
tumescens	NCIB8914*	Soil
ureafaciens	NCIB7811	Soil
sp.	NCIB9859	Poultry deep litter
sp.	NCIB9860	Poultry deep litter
Brevibacterium		
divaricatum	NCIB9379	Sewage
fermentans	NCIB9943*	Air
fuscum	NCIB9684	Soil
helvolum	NCIB9792	Dune Sand
linens	NCIB8546	Cheese
liquefaciens	NCIB9545*	Sewage
oxydans	NCIB9944*	Air
stationis	NCMB565	Marine

Table 1 (*cont.*)

Organism	Strain	Source
Mycobacterium		
rhodochrous	NCIB9259	Soil
rhodochrous	NCIB9703	Soil
rhodochrous	NCIB9784	Soil
smegmatis	NCIB8548	—
Nocardia		
calcarea	NCIB8863*	Chalk soil
cellulans	NCIB8868	Chalk soil
canicruria	NCIB8147*	Soil
Microbacterium		
flavum	NCIB8707*	Cheese
lacticum	NCIB8540*	Milk
Cellulomonas		
biazotea	NCIB8077	Soil
fimi	NCIB8980	—
Listeria monocytogenes	NCTC7973	Animal
Erysipelothrix rhusiopathiae	NCTC8163	Animal
Flavobacterium		
arborescens	NCMB549	Marine
dehydrogenans	NCMB872	—
dehydrogenans	NCMB873	—
marinotypicum	NCMB559	Marine
marinotypicum	NCMB689	Marine
marinotypicum	NCMB1050	Marine
sp.	NCMB867	—
sp.	NCMB868	—
sp.	NCMB869	—
sp.	NCMB870	—
sp.	NCMB871	—
sp.	NCMB874	—
'Coryneform'	NCIB9568	Soil
	NCIB9569	Soil
	NCIB9646	Soil
	NCIB9864	Poultry deep litter
	NCMB8	Fish
	NCMB12	Fish
	NCMB31	Fish
	NCMB32	Fish
	NCMB33	Fish
	NCMB34	Fish
	NCMB35	Fish
	NCMB280	Fish
	NCMB299	Marine
	NCMB312	Fish
	NCMB313	Fish
	NCMB314	Fish
	NCMB315	Fish
	NCMB316	Fish
	NCMB317	Fish
	NCMB477	Marine
	NCMB480	Marine
	NCMB497	Marine
	NCMB502	Marine
	NCMB533	Marine
	NCMB577	Marine
	NCMB598	Marine

Table 1 (*cont.*)

Organism	Strain	Source
'Coryneform' (*cont.*)	NCMB599	Marine
	NCMB1094	Fish
	NCMB1320	Marine
	NCMB1321	Marine
	NCMB1322	Marine
	NCMB1323	Marine
	NCMB1324	Marine
	AC4	—
	AC11	—
	AC166	—
	AC251	—
	AC252	—
	AC253	—
	AC254	—
	AC259	—
	AC260	—
	AC365	—
	AC366	—
	AC367	—
	C48A	Plant
	C48B	Plant
	C48D	Plant
	C97D	Plant
	IJBI-10	Marine
	IJBII-38	Soil

* Type strain.

are indicated by arabic numerals on the dendrogram. For convenient description in the text, certain clusters within subphenon IIB are indicated on the dendrogram by lower case letters.

The values obtained from DNA base composition determinations are given in Table 3.

Phenon II. Most of the 92 strains in this phenon were Gram-positive, pleomorphic, coryneform rods with a morphological cycle. With the exception of NCIB9646, those strains not showing a morphological cycle under the test conditions (*Mycobacterium smegmatis* NCIB8548, *M. rhodochrous* NCIB9259, NCIB9784 and NCIB9703, IJB28, IJB19 and *Corynebacterium fascians* NCPPB1488) are all in subphenon IIA. Variations in the Gram reaction at different ages of the cultures were common, although no definite pattern emerged. In only three cases (*Arthrobacter atrocyaneus* NCIB9220, *A. globiformis* NCIB8907 and *A. pascens* NCIB8910) did cells appear completely Gram-negative in very young cultures, becoming Gram-positive as the cultures aged. Only three strains (*M. smegmatis* NCIB8548, *M. rhodochrous* NCIB9259 and NCIB9784) were acid-fast by the method used. All strains in phenon II were strict aerobes and were able to utilize ammonium nitrogen. Most strains did not produce acid from a wide range of carbohydrates but were able to utilize acetate, pyruvate, citrate and lactate as sole carbon sources. Strains in subphenon IIA were generally feebly or non-proteolytic whereas those in subphenon IIB, with the exception of a few strains in cluster 3, were actively proteolytic. Members of IIB also appeared to utilize more amino acid as sole carbon sources than did members of IIA. With the exceptions of *M. smegmatis* NCIB8548 (70 % GC) and *Corynebacterium michiganense* NCPPB1468 (71 % GC) all strains tested in subphenon IIA showed % GC values in the range 61 to 66 and were indistinguishable by % GC values from strains in subphenon IIB, which showed values in the range 61 to 64%. This phenon contains all the named strains of *Nocardia, Mycobacterium* and *Arthrobacter* with the exceptions of *A. citreus* NCIB8915, *A. tumescens* NCIB8914 and *Nocardia cellulans* NCIB8868.

Table 2. *Individual features recorded for numerical analysis*

(D) Acid from adonitol	(D) Utilization of inorganic nitrogen
(D) Acid from arabinose	(D) Oxidase production
(D) Acid from cellobiose	(D) Catalase production
(D) Acid from dextrin	(D) H_2S production
(D) Acid from fructose	(D) Phosphatase production
(D) Acid from galactose	(D) Casein hydrolysis
(D) Acid from glucose	(D) Gelatin hydrolysis
(D) Acid from glycerol	(D) Starch hydrolysis
(D) Acid from inulin	(D) Urea hydrolysis
(D) Acid from inositol	(D) Voges–Proskauer test
(D) Acid from lactose	(D) Methyl red test
(D) Acid from maltose	(A) Penicillin (sensitive/resistant)
(D) Acid from mannitol	(D) Anaerobic growth in glucose
(D) Acid from mannose	(D) NO_3 reduction
(D) Acid from raffinose	(D) Litmus milk-reduction
(D) Acid from rhamnose	(D) Litmus milk-acid
(D) Acid from ribose	(D) Litmus milk-proteolysis
(D) Acid from salicin	(D) Litmus milk-clot
(D) Acid from sorbitol	(D) Cellulose decomposition
(D) Acid from sorbose	(Q) NaCl tolerance (2·5 %/5 %/7·5 %/10 %)
(D) Acid from sucrose	(Q) Growth temperatures – 'high' (30°/37°/45 °C)
(D) Acid from trehalose	(Q) Growth temperatures – 'low' (0·6°/10°/20 °C)
(D) Acid from xylose	(D) Heat resistance
(D) Utilization of acetate	(D) Rods
(D) Utilization of citrate	(D) Cocci
(D) Utilization of lactate	(D) Cocco-bacilli
(D) Utilization of hippurate	(D) Clubs
(D) Utilization of formate	(D) 'Chinese letters'
(D) Utilization of oxalate	(D) V-forms
(D) Utilization of succinate	(D) Filaments
(D) Utilization of malate	(D) Palisades
(D) Utilization of fumarate	(D) 'Cystites'
(D) Utilization of malonate	(D) 'Adderheads'
(D) Utilization of tartrate	(Q) Length of rods
(D) Utilization of pyruvate	(Q) Thickness of rods
(D) Utilization of mandelate	(D) Granules in cells
(D) Utilization of benzoate	(D) Branching
(D) Utilization of phenol	(D) 'Life-cycle'
(D) Utilization of alanine	(A) Gram-stain 6 h (+/±/−)
(D) Utilization of serine	(A) Gram-strain 24 h (+/±/−)
(D) Utilization of threonine	(A) Gram-strain 48 h (+/±/−)
(D) Utilization of leucine	(D) Acid fastness
(D) Utilization of valine	(D) Motility
(D) Utilization of glutamate	(A) Colony surface (rough/smooth/mucoid)
(D) Utilization of lysine	(A) Colony elevation (flat/convex)
(D) Utilization of arginine	(A) Colony pigment (cream/yellow/orange/pink)
(D) Utilization of ornithine	(A) Colony margin (entire/irregular)
(D) Utilization of γ-aminobutyrate	(A) Colony opacity (translucent/opaque)
(D) Utilization of histidine	(Q) Colony size
(D) Utilization of proline	(D) Surface ring in broth
(D) Utilization of tyrosine	(D) Pellicle in broth
(D) Utilization of phenylalanine	(A) Sediment in broth (floccose/pellet)
(D) Utilization of cystine	(Q) Turbidity in broth
(D) Utilization of cysteine	(D) Production of fluorescent pigment
(D) Utilization of methionine	(A) Colour of fluorescent pigment (red/green)

NB. Since all results for production of indole and production of acid from dulcitol were negative, these tests were omitted from the computation. (A) = Alternative, (D) = dichotomy, (Q) = quantitative. See text for explanation.

Table 3. T_m *and* % *GC values of selected strains*

All %GC values are given to the nearest 0·5 %.

Strain		Phenon	T_m (°C)	%GC
IJB30*		II	95·3	63·5
NCMB280*		II	96·4	66
NCIB8548	*Mycobacterium smegmatis*	II	97·9	70
NCIB9259	*M. rhodochrous*	II	96·4	66
IJB28*		II	94·9	62·5
NCIB8863	*Nocardia calcarea*	II	94·9	62·5
NCIB8147	*N. canicruria*	II	94·6	61·5
NCIB9646*		II	94·8	62
NCPPB1488	*Corynebacterium fascians*	II	94·2	61
NCIB8546	*Brevibacterium linens*	II	94·4	61
NCMB1324*		II	94·9	62·5
NCMB12*		II	95·6	64
NCIB8907	*Arthrobacter globiformis*	II	95·3	63·5
NCIB7811	*A. ureafaciens*	II	94·8	62
NCIB8910	*A. pascens*	II	95·3	63·5
NCIB9792	*Brevibacterium helvolum*	II	94·3	61
NCPPB1228	*Corynebacterium ilicis*	II	94·6	61·5
IJB35*		II	94·6	61·5
NCIB9545	*Brevibacterium liquefaciens*	II	94·8	62
NCIB9684	*B. fuscum*	II	94·8	62
NCMB31*		II	94·6	61·5
NCMB35*		II	94·6	61·5
NCIB9569*		II	94·8	62
NCPPB1468	*Corynebacterium michiganense*	II	98·5	71
NCPPB559	*C. flaccumfaciens*	II	97·2	68
NCPPB844	*C. poinsettiae*	II	97·9	70
NCMB5	*C. erythrogenes*	II	94·3	61
NCMB1050	*Flavobacterium marinotypicum*	III	96·1	65·5
NCIB9944	*Brevibacterium oxydans*	III	96·9	67
NCIB8980	*Cellulomonas fimi*	III	96·4	66
NCMB871	*Flavobacterium* sp.	III	96·4	66
NCMB868	*Flavobacterium* sp.	III	96·3	66
NCIB7206	*Corynebacterium mediolanum*	III	96·3	66
NCIB8540	*Microbacterium lacticum*	III	98·1	70
C48A*		III	93·6	59
NCMB670*		III	99·0	73
NCIB8077	*Cellulomonas biazotea*	III	98·6	71·5
NCIB8868	*Nocardia cellulans*	III	98·1	70
NCIB 9943	*Brevibacterium fermentans*	III	99·1	73
NCIB9097	*Corynebacterium manihot*	III	98·1	70
NCIB9859*		III	96·3	66
NCIB9860*		III	96·4	66
NCTC7429	*Corynebacterium diphtheriae*	IV	92·3	55
NCTC9755	*C. xerosis*	IV	93·6	59
NCPPB363	*C. betae*	IV	96·5	66·5
NCIB9379	*Brevibacterium divaricatum*	IV	90·2	51
NCIB9694	*Corynebacterium herculis*	IV	90·3	51
NCIB9661	*C. acetoacidophilum*	IV	90·3	51
NCIB8707	*Microbacterium flavum*	IV	92·8	56

* These strains have not been formally identified and are therefore not given names.

Phenon III. Most of the 36 strains in this phenon were slender, Gram-positive or Gram-variable coryneform rods (and occasionally filaments) breaking down into cocco bacilli in 48 to 72 h. Individual cells were generally smaller than those found in strains of phenon II and they stained less deeply by the Gram-reaction. No strains were acid-fast by the method used. Motility was quite often encountered in this phenon, but no pattern was apparent.

All strains in subphenon IIIA were strict aerobes and were able to utilize ammonium nitrogen. Most strains produced acid from a fairly wide range of carbohydrates and were able to utilize acetate, lactate, pyruvate and glutamate as sole carbon sources. All the so-called Flavobacterium strains in cluster 6 were able to withstand heating at 60 °C for 30 min. All strains in subphenon IIIB with the exceptions of NCIB9860 and IJB15 were facultatively anaerobic. Most strains readily produced acid from a wide range of carbohydrates and were able to utilize ammonium nitrogen. The ability to utilize various compounds as sole carbon sources was much less pronounced than in subphenon IIIA. Three strains (*Cellulomonas biazotea* NCIB8077, *Nocardia cellulans* NCIB8868 and IJB11) were cellulolytic. The % GC values for strains tested in subphenon IIIA were in the narrow range 65 to 67, whereas the range of % GC values in subphenon IIIB was much wider. The value obtained for the single strain tested in cluster 8 (C48A) was 59% and that obtained for NCIB9859 and NCIB9860 was 66%. The % GC values for all other strains tested in this subphenon (*C. biazotea* NCIB8077, *N. cellulans* NCIB8868, *Brevibacterium fermentans* NCIB9943, *Corynebacterium manihot* NCIB9097, *Microbacterium lacticum* NCIB8540 and NCMB670) were in the range 70 to 73%.

Phenon IV. Most strains in this phenon were Gram-positive or Gram-variable coryneform rods of various sizes. With the exception of *Corynebacterium ovis* NCTC3450 and *Brevibacterium divaricatum* NCIB9379 a morphological cycle was not noted. *Listeria monocytogenes* NCTC7973 and *Erysipelothrix rhusiopathiae* NCTC8163 were morphologically exceptional in that neither strain showed any of the usual coryneform features. The former showed straight, regular rods whilst the latter showed loops, 'horseshoes' and filaments. Neither of these strains showed any morphological similarity to any other strain included in the present study. All strains with the exception of *C. betae* NCPPB363 were facultatively anaerobic. Strains in subphenon IVB utilized ammonium nitrogen under the test conditions, whereas those in subphenon IVA did not. All strains in subphenon IVB with the exception of *Microbacterium flavum* NCIB8707 were able to tolerate heating at 63 °C for 30 min by the method of testing. The strains in subphenon IVA tested for DNA base composition (*C. betae* NCPPB363, *C. diphtheriae* NCTC7429 and *C. xerosis* NCTC9755) had % GC values of 66·5, 55 and 59 respectively. The % GC value for *M. flavum* NCIB8707 was 56; that for the other three strains in subphenon IVB was 51.

Phenon V. The six strains in this small phenon were Gram-positive or Gram-variable, pleomorphic, coryneform rods. No morphological cycle was observed in any of the strains in this phenon. All strains were strictly aerobic and were able to utilize ammonium nitrogen. Most of the sugar fermentation and carbon utilization tests gave negative results.

DISCUSSION

In general, our results are in agreement with those obtained in similar studies by other workers which have also indicated that some redistribution of strains in the various coryneform genera appears necessary. To this end, each major phenon will be discussed in turn and its status in the context of current coryneform genera will be considered.

Phenon II. This phenon represents *Nocardia/Mycobacterium* and *Arthrobacter*. Most strains in IIA were similar to the *Mycobacterium rhodochrous* group of organisms described by Gordon (1966). None of the strains in IIA appeared to be closely related to the animal pathogenic corynebacteria of phenon IV, which supports the conclusion of Davis & Newton (1969) that despite earlier suggestions to the contrary (Cummins 1962; Harrington, 1966), *Mycobacterium* and *Nocardia* should remain separate from *Corynebacterium*.

The inclusion of *Corynebacterium fascians* in IIA supports the already considerable body of evidence that this organism is a nocardia (Lacey, 1955; Ramamurthi, 1959) or at least a member of the *Mycobacterium rhodochrous* group (Gordon (1966), who discussed the difficulties of assigning the latter group to a suitable genus).

Corynebacterium michiganense NCPPB1468 is placed in subphenon IIA, whereas Da Silva & Holt (1965) and Davis & Newton (1969) found that the strains of *C. michiganense* used by them clustered with *Microbacterium lacticum*. The single strain of *M. lacticum* (NCIB8540) used in the present work is placed (unclustered) in subphenon IIIB.

All strains in IIB showed the morphological 'life-cycle' of 'typical' arthrobacters. This phenomenon appears to be the principal feature of the genus *Arthrobacter* as originally defined by Conn & Dimmick (1947), and Skerman (1967) emphasizes that, in the identification of *Arthrobacter*, the final transformation into cocci is the most important feature to be observed. *Bergey's Manual of Determinative Bacteriology* (Breed, Murray & Smith, 1957) also stresses this point. Whilst such a morphological cycle may be a prerequisite for the genus *Arthrobacter*, our results show that it is by no means restricted to this genus. Indeed, its occurrence in the coryneform group seems to be the rule rather than the exception (Veldkamp, 1970) and it has been observed in many coryneform organisms including *Cellulomonas* and *Corynebacterium diphtheriae*, albeit less strikingly than in 'typical' arthrobacters (Ørskov, 1923; Grubb & Koser, 1934; Jensen, 1952; Gibson, 1953; Müller, 1957; Kuhn & Starr, 1962; Mulder & Antheunisse, 1963). Keddie *et al.* (1966) commented upon the unreliability of the 'life-cycle' as a definitive criterion for the recognition of Arthrobacter species, concluding that the creation of a separate genus on morphological grounds alone was not justified. Thus *Arthrobacter* must be defined by more suitable criteria than the mere occurrence of a morphological cycle. In this context, the study of nutritional features and cell-wall composition may prove useful in the future (Keddie, Leask & Grainger, 1966; Owens & Keddie, 1968, 1969; I. J. Bousfield, unpublished results).

In subphenon IIB, cluster c appears to represent the *Arthrobacter globiformis* type of coryneform. Included in cluster c are *Brevibacterium helvolum* NCIB9792, *B. liquefaciens* NCIB9545, *B. fuscum* NCIB9684 and *Corynebacterium ilicis* NCPPB1228, all of which are very similar to *A. globiformis* and all of which would be more suitably accommodated in the genus *Arthrobacter* than in either of *Brevibacterium* or *Corynebacterium*. Cluster b consists of *Brevibacterium linens* NCIB8546 and similar strains. The similarity of *B. linens* to members of *Arthrobacter* has already been noted by several workers (e.g. Schefferle, 1957; Mulder & Antheunisse, 1963; Mulder, 1964; Mulder *et al.* 1966) and Da Silva & Holt (1965) and Davis & Newton (1969) suggested that it be renamed *Arthrobacter linens*. If the results of previous work are taken with those obtained in the present study, it would seem that *B. linens*, whilst differing in several respects from *A. globiformis*, should nevertheless be contained in the same genus as the latter.

The inclusion of cluster a in subphenon IIB is of note since this cluster includes named strains of *Nocardia canicruria* and *N. calcarea*, organisms considered by Gordon (1966) to belong to the *Mycobaterium rhodochrous* group. The strains in cluster a are, in fact, linked to many of the strains in subphenon IIA as can be seen from the S-matrix in Fig. 1. These linkages cannot be satisfactorily indicated on the dendrogram in Fig. 2. However, the various linkages shown by the strains of cluster a tend to emphasize the indeterminate nature of the boundary between *Arthrobacter* and *Nocardia*.

Cluster d consists of unnamed arthrobacter-like soil strains and cluster e consists of unnamed marine organisms linked to a further four marine strains including *Corynebacterium erythrogenes* NCMB5, which again would seem to be more suitably placed in *Arthrobacter*

than in *Corynebacterium*. *Corynebacterium barkeri* NCIB9658, which is amongst the unclustered strains in subphenon IIB, also seems to resemble the arthrobacters more than the corynebacteria.

Phenon III. Most of the strains in subphenon IIIA were originally designated Flavobacterium strains, but it seems obvious that these so-called flavobacteria are coryneforms. The similarity of the Flavobacterium strains of group 6 to *Corynebacterium mediolanum* NCIB7206 is enhanced by their cell wall composition (Bousfield, 1969). The cell walls of all the strains in group 6 contain diaminobutyric acid as the principal diamino acid. This compound was previously reported in the cell wall of *Corynebacterium tritici* (Perkins & Cummins, 1964).

The strains in subphenon IIIA could be regarded as all belonging to the same genus, since in addition to their overall similarity the % GC range is fairly narrow (65 to 67), but selection of a suitable genus remains a problem. These strains were sufficiently distinct in this study to warrant their exclusion from all of the existing coryneform genera. The only previously described genus into which the strains of subphenon IIIA can be easily fitted is *Brevibacterium* (Chatelain & Second, 1966). One of the strains of Chatelain & Second (*Brevibacterium oxydans* NCIB9944) is included in subphenon IIIA. However, *Brevibacterium* (Chatelain & Second, 1966) is illegitimate as it does not include *B. linens*, the type species of the genus *Brevibacterium* (Breed, 1953*a*, *b*) which was legitimately described. Therefore, the creation of a new genus would seem to be needed.

Included in subphenon IIIA is a strain of *Cellulomonas fimi* (NCIB8980), an organism which seems to be wrongly classified since it is non-cellulolytic and shows very little relationship with *Cellulomonas biazotea*. Also included in subphenon IIIA is *Corynebacterium rubrum* NCIB9433, which according to Gordon (1966) is similar to *Mycobacterium rhodochrous*, but this similarity has not been demonstrated in the present work.

In subphenon IIIB the strains of group 7 (*Nocardia cellulans* NCIB8868, *Brevibacterium fermentans* NCIB9943, IJB11, *Corynebacterium manihot* NCIB9097 and *Cellulomonas biazotea* NCIB8077) have a similar DNA base composition and they appear to be related in terms of overall similarity. In addition, *Nocardia cellulans* and IJB11 are cellulolytic. It seems possible that these strains could all belong to the same genus. However, M. Goodfellow (personal communication) found that *Nocardia cellulans* showed some similarity to *Oerskovia turbata* (Prauser, Lechevalier & Lechevalier, 1970), the relationship of which to *Nocardia cellulans* and other organisms is currently under investigation in this laboratory.

Phenon IV. With the exception of three strains (*Corynebacterium betae* NCPPB363, *Listeria monocytogenes* NCTC7973 and *Erysipelothrix rhusiopathiae* NCTC8163) subphenon IVA consists of animal pathogenic corynebacteria. These organisms have generally been considered closely related to each other and the present results bear this out. The % GC values obtained for two strains (*Corynebacterium diphtheriae* NCTC7429, 54% and *C. xerosis* 59% NCTC7755) are fairly close and fall into the % GC range for corynebacteria determined by Bouisset *et al.* (1963). However, coryneform DNA base analysis by Yamada & Komagata (1970*b*) casts some doubt both on the results of Bouisset *et al.* (1963) and on the apparently close interrelationships amongst the animal pathogenic corynebacteria. Yamada & Komagata (1970*b*) gave a % GC range of 52 to 68 for the animal pathogenic corynebacteria, whereas the range given by Bouisset *et al.* (1963) was 52 to 59.

It may be noted that *Corynebacterium equi* NCTC1621 falls into phenon IV and that a relationship between this organism and the mycobacteria (Jensen, 1934, 1952; Harrington, 1966) has not been demonstrated here. Although *Erysipelothrix rhusiopathiae* NCTC8136 and *Listeria monocytogenes* NCTC7973 are included in phenon IV, their relationships with

the coryneform bacteria appears doubtful. Neither organism shows coryneform morphology and the % GC value for *L. monocytogenes* (38%; Marmur & Doty, 1962) is well outside the coryneform range. It has been suggested that *Listeria* and *Erysipelothrix* are more closely related to the family Lactobacillaceae than to the family Corynebacteriaceae (Davis *et al.* 1969).

The four organisms in subphenon IVB (*Microbacterium flavum* NCIB8707, *Corynebacterium herculis* NCIB9694, *C. acetoacidophilum* NCIB9661 and *Brevibacterium divaricatum* NCIB9379) are very similar to each other and in the similarity matrix appear to be linked to the strains of group 7 in subphenon IIIB. Previous reports (e.g. Robinson, 1966*a*) indicate that *M. flavum* should belong to the same genus as the animal pathogenic corynebacteria and the % GC values for strains in subphenon IVB (51 to 56 %) are fairly close to that of *Corynebacterium diphtheriae* (52 to 54%). Whilst these organisms are in the same phenon a close relationship has not been demonstrated here.

Phenon V. With the exception of IJB37, the strains in this phenon are plant-pathogenic Corynebacterium species. Since most strains gave negative results for many of the physiological tests, it is likely that this phenon has been formed largely on the basis of morphological similarities. The plant pathogenic corynebacteria in this phenon seem to be sufficiently distinct from the 'true' corynebacteria of phenon IV to warrant their exclusion from the genus *Corynebacterium* but they do not fit readily into any other genus.

General conclusions

The main points brought out in the present work may be summarized as follows.

(1) The genus *Corynebacterium* is obviously unsatisfactory as it stands at present. Whilst the animal pathogens may form a satisfactory group, other named *Corynebacterium* spp. are widely divergent. There may well be a case for restricting the genus *Corynebacterium* to certain animal pathogens and related types and for placing all other members of the genus elsewhere.

(2) The taxonomy of *Brevibacterium* is not satisfactory. From its inception (Breed, 1953*a*, *b*), *Brevibacterium* was never more than a repository for a variety of Gram-positive organisms and as a defined genus it seems to have little value. The type species (*Brevibacterium linens*) is probably better placed in the genus *Arthrobacter*, thus rendering *Brevibacterium* invalid on nomenclatural grounds alone. A new concept of *Brevibacterium* was described illegitimately by Chatelain & Second (1966) and the present work supports (in some measure) the contention of these workers that such a group of coryneforms exists and may well merit generic status. However, nomenclatural rules preclude the use of the name *Brevibacterium* for this group.

(3) Several organisms which were previously classified as flavobacteria would seem to be better placed in the coryneform group. These strains form the nucleus of the group mentioned in (2) above.

(4) Certain non-cellulolytic strains appear to be similar to *Cellulomonas biazotea* and could possibly be included in the same genus. *Nocardia cellulans* would also probably be better placed in *Cellulomonas* than in *Nocardia.*

(5) The genus *Arthrobacter* seems to be more homogeneous than many other coryneform genera and forms an admirable repository for many of the saprophytic diphtheroids and for certain aberrant strains from other coryneform genera. However, the boundary between *Arthrobacter* and *Nocardia* is ill-defined and several organisms of the *Mycobacterium rhodochrous* type seem to be transitional between the two genera.

(6) The unsatisfactory nature of the genus *Microbacterium* has again been shown but the results have not provided a solution.

The author wishes to acknowledge the receipt of a Natural Environment Research Council grant during the course of this work. This paper was prepared as part of the programme of the Torry Research Station.

REFERENCES

ABE, S., TAKAYAMA, K. & KINOSHITA, S. (1967). Taxonomical studies on glutamic acid-producing bacteria. *Journal of General and Applied Microbiology* **13**, 279–301.

ABD-EL-MALEK, Y. & GIBSON, T. (1952). Studies in the bacteriology of milk. III. The corynebacteria of milk. *Journal of Dairy Research* **19**, 153–159.

BOUISSET, L., BREUILLARD, J. & MICHEL, G. (1963). Étude de l'ADN chez les *Actinomycetales*. *Annales de l'Institut Pasteur* **104**, 756–770.

BOUSFIELD, I. J. (1969). *A Taxonomic Study of Some Aerobic Coryneform Bacteria.* PhD Thesis, University of Aberdeen.

BREED, R. S. (1953*a*). The Brevibacteriaceae fam.nov. of order *Eubacteriales*. *Abstracts of Communications, 6th International Congress of Microbiology, Rome* **1**, 13–14.

BREED, R. S. (1953*b*). The families developed from Bacteriaceae Cohn with a description of the family Brevibacteriaceae Breed 1953. *Proceedings of 6th International Congress of Microbiology, Rome* **1**, 10–15.

BREED, R. S., MURRAY, E. G. D. & SMITH, N. R. (1957). *Bergey's Manual of Determinative Bacteriology*, 7th edn. London: Baillère, Tindall & Cox.

CHATELAIN, R. & SECOND, L. (1966). Taxonomie numérique de quelques *Brevibacterium*. *Annales de l'Institut Pasteur* **111**, 630–644.

CLARK, F. E. (1952). The generic classification of the soil corynebacteria. *International Bulletin of Bacterial Nomenclature and Taxonomy* **2**, 45–56.

CONN, H. J. & DIMMICK, I. (1947). Soil bacteria similar in morphology to *Mycobacterium* and *Corynebacterium*. *Journal of Bacteriology* **54**, 291–303.

CUMMINS, C. S. (1962). Immunochemical specificity and the location of antigens in the bacterial cell wall. In *Microbial Classification, Symposium of the Society for General Microbiology* **12**, 212–241.

DA SILVA, G. A. N. & HOLT, J. G. (1965). Numerical taxonomy of certain coryneform bacteria. *Journal of Bacteriology* **90**, 921–927.

DAVIS, G. H. G., FOMIN, L., WILSON, E. & NEWTON, K. G. (1969). Numerical taxonomy of *Listeria*, streptococci and possibly related bacteria. *Journal of General Microbiology* **57**, 333–348.

DAVIS, G. H. G. & NEWTON, K. G. (1969). Numerical taxonomy of some named coryneform bacteria. *Journal of General Microbiology* **56**, 195–214.

GIBSON, T. (1953). The taxonomy of the genus *Corynebacterium*. *Proceedings of 6th International Congress of Microbiology, Rome* **1**, 16–20.

GORDON, R. E. (1966). Some strains in search of a genus – *Corynebacterium, Mycobacterium* or what? *Journal of General Microbiology* **43**, 329–343.

GRUBB, T. C. & KOSER, S. A. (1934). Coccus forms of *Corynebacterium diphtheriae*. *Journal of Bacteriology* **27**, 45.

HARRINGTON, B. J. (1966). A numerical taxonomical study of some corynebacteria and related organisms. *Journal of General Microbiology* **45**, 31–40.

JENSEN, H. L. (1934). Studies on the saprophytic mycobacteria and corynebacteria. *Proceedings of the Linnean Society of New South Wales* **59**, 19–61.

JENSEN, H. L. (1952). The coryneform bacteria. *Annual Review of Microbiology* **6**, 77–90.

JENSEN, H. L. (1953). The genus *Nocardia* (or *Proactinomyces*) and its separation from other *Actinomycetales*, with some reflections on the phylogeny of the actinomycetes. *6th International Congress of Microbiology, Rome* **1**, 69–88.

JENSEN, H. L. (1966). Some introductory remarks on the coryneform bacteria. *Journal of Applied Bacteriology* **29**, 13–16.

KEDDIE, R. M., LEASK, B. G. S. & GRAINGER, J. M. (1966). A comparison of coryneform bacteria from soil and herbage: cell wall composition and nutrition. *Journal of Applied Bacteriology* **29**, 17–43.

KOMAGATA, K., YAMADA, K. & OGAWA, H. (1969). Taxonomic studies on coryneform bacteria. I. Division of bacterial cells. *Journal of General and Applied Microbiology* **15**, 243–259.

KOVACS, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature, London* **178**, 703.

KUHN, D. A. & STARR, M. P. (1962). Developmental morphology of *Corynebacterium poinsettiae*. *Bacteriological Proceedings*, 46.

LACEY, M. S. (1955). The cytology and relationships of *Corynebacterium fascians*. *Transactions of the British Mycological Society* **38**, 49–58.

MARMUR, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *Journal of Molecular Biology* **3**, 208–218.

MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.

MASUO, E. & NAKAGAWA, T. (1969). Numerical classification of bacteria. Part II. Computer analysis of coryneform bacteria: comparison of group-formations obtained on two different methods of scoring data. *Agricultural and Biological Chemistry* **33**, 1124–1133.

MULDER, E. G. (1964). Arthrobacter. In *Principles and Applications in Aquatic Microbiology*. Edited by H. Heukelekian & N. Dondero. New York: John Wiley & Sons.

MULDER, E. G., ADAMSE, A. D., ANTHEUNISSE, J., DEINEMA, M. H., WOLDENDORP, J. W. & ZEVENHUIZEN L. P. T. M. (1966). The relationship between *Brevibacterium linens* and bacteria of the genus *Arthrobacter*. *Journal of Applied Bacteriology* **29**, 44–71.

MULDER, E. G. & ANTHEUNISSE, J. (1963). Morphologie, physiologie et écologie des *Arthrobacter*. *Annales de l'Institut Pasteur* **105**, 46–74.

MULLAKHANBHAI, M. F. & BHAT, J. V. (1967). A numerical taxonomical study of *Arthrobacter*. *Current Science* **36**, 115–118.

MÜLLER, J. (1957). Untersuchungen zur Morphologie und Physiologie der Corynebakterien. *Archiv für Mikrobiologie* **27**, 105–124.

ØRSKOV, J. (1923). *Investigations into the Morphology of the Ray Fungi*. Copenhagen: Levin & Munksgaard.

OWENS, J. D. & KEDDIE, R. M. (1968). A note on the vitamin requirements of some coryneform bacteria from soil and herbage. *Journal of Applied Bacteriology* **31**, 344–348.

OWENS, J. D. & KEDDIE, R. M. (1969). The nitrogen nutrition of soil and herbage coryneform bacteria. *Journal of Applied Bacteriology* **32**, 338–347.

PERKINS, H. R. & CUMMINS, C. S. (1964). Chemical structure of bacterial cell walls. Ornithine and 2-4 diaminobutyric acid as components of the cell walls of plant pathogenic corynebacteria. *Nature, London* **201**, 1105–1107.

PRAUSER, H., LECHEVALIER, M. P. & LECHEVALIER, H. (1970). Description of *Oerskovia* gen. n. to harbour Ørskov's motile *Nocardia*. *Applied Microbiology* **19**, 534.

RAMAMURTHI, C. S. (1959). Comparative studies on some Gram-positive phytopathogenic bacteria and their relationship to the corynebacteria. *Memorandum of Cornell University Agricultural Experimental Station, no.* 366. Ithaca, New York: New York State College of Agriculture.

ROBINSON, K. (1966*a*). *A Study of Coryneform Bacteria with Particular Reference to the Genus Microbacterium*. Ph.D. Thesis, University of Aberdeen.

ROBINSON, K. (1966*b*). Some observations on the taxonomy of the genus *Microbacterium*. I. Cultural and physiological reactions and heat resistance. *Journal of Applied Bacteriology* **29**, 607–615.

ROBINSON, K. (1966*c*). Some observations on the taxonomy of the genus *Microbacterium*. II. Cell wall analysis, gel electrophoresis and serology. *Journal of Applied Bacteriology* **29**, 616–624.

ROBINSON, K. (1966*d*). An examination of *Corynebacterium* species by gel electrophoresis. *Journal of Applied Bacteriology* **29**, 179–184.

SCHEFFERLE, H. E. (1957). *An Investigation of the Microbiology of Built-up Poultry Litter*. Ph.D. Thesis, University of Edinburgh. Cited in Mulder *et al.* (1966).

SKERMAN, V. B. D. (1967). *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.

SKYRING, G. W. & QUADLING, C. (1970). Soil bacteria: principal component analysis and guanine-cytosine contents of some arthrobacter-coryneform soil isolates and of some named cultures. *Canadian Journal of Microbiology* **16**, 95–106.

SOCIETY OF AMERICAN BACTERIOLOGISTS (1957). *Manual of Microbiological Methods*. Edited by H. J. Conn. London: McGraw Hill Book Co.

SPLITSTOESSER, D. E., WEXLER, M., WHITE, J. & COLWELL, R. R. (1967). Numerical taxonomy of Gram-positive and catalase-positive rods isolated from frozen vegetables. *Applied Microbiology* **15**, 158–162.

STANIER, R. Y., PALLERONI, N. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* **43**, 159–271.

VELDKAMP, H. (1970). Saprophytic coryneform bacteria. *Annual Review of Microbiology* **24**, 209–240.

WILSON, G. S. & MILES, A. A. (1964). *Topley and Wilson's Principles of Bacteriology and Immunity*, vol. 1, 5th edn. London: Arnold.

YAMADA, K. & KOMAGATA, K. (1968). Taxonomic studies on coryneform bacteria. *International Conference of Culture Collections Tokyo (Abstr.)*, p. 23. Tokyo: University of Tokyo Press.

YAMADA, K. & KOMAGATA, K. (1970*a*). Taxonomic studies on coryneform bacteria. II. Principal amino acids in the cell wall and their taxonomic significance. *Journal of General and Applied Microbiology* **16**, 103–113.

YAMADA, K. & KOMAGATA, K. (1970*b*). Taxonomic studies on coryneform bacteria. III. DNA base composition of coryneform bacteria. *Journal of General and Applied Microbiology* **16**, 215–224.