Numerical Taxonomy of Some Nocardioform Bacteria

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SUMMARY

A collection of 283 nocardioform bacteria was studied by means of 241 tests which included many biochemical, physiological and nutritional characters. The results were subjected to computer analysis in which two coefficients of association and two cluster-analysis techniques were used. Single linkage and average linkage cluster analysis gave almost identical results, but with the simple matching coefficient a few strains were grouped together on the basis of shared negative correlations. With the matching coefficient 15 clusters were formed whereas with the similarity coefficient there were 18. In each analysis the seven major clusters corresponded to (1) *Nocardia asteroides*, (2) *Nocardia caviae*, (3) *Nocardia brasiliensis*, (4) *Actinomadurea madurae*, (5) *Actinomadurea pelletieri*, (6) *Oerskovia turbata*, (7) the rhodochrous complex. The data revealed many new characters which could be weighted for identification. The clusters and subgroups were therefore readily distinguished from one another by several unrelated phenotypic characters.

INTRODUCTION

Bacteria which produce a mycelium that fragments into rods and cocci have been referred to by the term nocardioform (Prauser, 1967). Many of these organisms are classified in the genus *Nocardia*, others as species of *Mycobacterium*. Nevertheless, great difficulty is found in characterizing nocardioform bacteria as so few good characters are available for identification. This paper presents detailed taxonomic information which shows that a large number of nocardioform organisms fall into only a few clusters, readily defined by several unrelated phenotypic characters. Some of the clusters appeared to warrant recognition as genera, others as species.

No taxonomist would consider the genus *Nocardia* to be well defined. The introduction to the genus in the 7th edition of *Bergey's Manual of Determinative Bacteriology* (1957) is long, imprecise and rests heavily on the assumption that nocardiae form a well-defined mycelium which, sooner or later, fragments completely. Bacteriologists and mycologists have assigned to *Nocardia* all kinds of bacteria which exhibit fragmenting mycelia, often without regard to other criteria. Fragmentation is not always easy to recognize and may often be a strain characteristic. It is not surprising, therefore, that the genus is heterogeneous and contains species which should be transferred to such established genera as *Micromonospora*, *Mycobacterium* and *Streptomyces* or be included in the recently proposed genera *Actinomadurea* (Lechevalier & Lechevalier, 1970) and *Oerskovia* (Prauser, Lechevalier & Lechevalier, 1970). It is clear that the genus requires drastic taxonomic revision and that future changes need to be based upon evidence more substantial than that used in the past.

Forty-four species of *Nocardia* are described in the last edition of *Bergey's Manual* (1957).

Many of the species are inadequately defined and differentiation is difficult if not impossible. In diagnostic keys a lot of weight is given to acid-fastness, to pigmentation and to morphological characters, properties considered to have a diagnostic value for the genera *Mycobacterium* and *Streptomyces*. With the nocardiae these characters have frequently been shown to be unsuitable, and reliance on them has tended to confuse rather than clarify the classification of the genus. Gordon (1966*a*) and Gordon & Mihm (1957, 1959*a*, 1962*a, b*) have developed a battery of tests to define and differentiate between the five well-known pathogenic species of *Nocardia*, and they reduced a number of nomenspecies to synonyms of these. The intraspecific variation patterns and the quantitative relationships between even these better-known species remain obscure.

The variability that nocardiae show towards some of the biochemical and staining tests encouraged the application of other techniques in attempts to resolve the taxonomic difficulties. Representative strains have been used in phage-typing and serological studies (Prauser & Falta, 1968; Kwapinski, 1970), in wall composition studies (Yamaguchi, 1965; Lechevalier & Lechevalier, 1971), and in the study of whole organism lipid patterns (Mordarska & Mordarski, 1969; Lechevalier, Horan & Lechevalier, 1971). The results of studies such as these have allowed the separation of nocardiae into broad groups of related strains but a reliable classification of the genus remains to be generally accepted. It was for this reason that an extensive comparative study of a large number of phenotypic characters of nocardiae and closely related organisms was undertaken.

In systematic studies it is important that sufficient strains of each taxon be taken to provide reasonable assurance that specific patterns of reactions will be detected. A list of all the legitimately described species of *Nocardia* was therefore compiled (Buchanan, Holt & Lessel, 1966), and the test material included where possible the type strain of each species, and organisms thought to represent the range of variation shown within the species. It was not possible to obtain cultures of 13 of the species described in *Bergey's Manual* (1957). In addition to the named strains, nocardiae isolated from soil, silage, sea and fresh water, and other nocardioform bacteria of uncertain taxonomic status were studied. Prominent amongst the latter were strains received as *Mycobacterium rhodochrous* (Gordon, 1966*b*). Finally, marker strains representing genera taxonomically related to *Nocardia* were included for comparison.

In studies with diverse groups of bacteria there is the problem of the general relevance of characters. In this study, group formation was based upon as many good characters as it was practical to handle. It was hoped that the 241 characters chosen reflected the spectrum of an organism's potential phenotypic attributes. Several characters, notably some chemotaxonomic ones, known to have a diagnostic value (Lechevalier & Lechevalier, 1971) were not studied because of the difficulty in collecting such data from a large number of bacteria. A few of the classical tests were included for continuity, and so were some of the characters found useful by Gordon (1967). There was, however, no conscious bias in test selection. Many of the tests were applied to nocardioform bacteria for the first time or, if used previously, had only been studied with a few strains. In particular, the bacteria were screened to see if they could use a range of sole carbon sources for their growth and energy requirements. Like pseudomonads, nocardioform bacteria are thought to be nutritionally versatile and able to use a wide range of organic compounds for their energy requirements (McClung, 1954; Clark & Hittle, 1962). Recently Raymond, Jamison & Hudson (1967) observed that nocardiae can oxidize methyl-substituted mono and dicyclic aromatic hydrocarbons, and Jones & Edington (1968) isolated nocardiae from soil which were able to oxidize *n*-octane, *n*-dodecane and cyclohexane. The inclusion of such nutritional tests was considered to be

justified because, as with the pseudomonads (Stanier, Palleroni & Doudoroff, 1966), some of these characters may have an important diagnostic value.

All the characters studied were given equal weight and the strains were classified on the basis of general similarity. Since nocardioform bacteria have not been subjected to an exhaustive systematic comparative study, there is no evidence on which to build a case for differential weighting. In a preliminary limited numerical taxonomic study I found that it was possible to detect clusters of nocardiae which could be defined by a few independent and highly correlated characters. During the course of this study other computer analyses based on Adansonian principles have been applied to the genus *Nocardia* with varying degrees of success (Cerbón, 1967; Tsukamura, 1969). An extended numerical taxonomic study in which the characters were given equal weight was therefore considered justified.

It was hoped that improved classification of the nocardioform bacteria would lead to the exclusion from the genus *Nocardia* of taxonomically unrelated forms and leave a homogeneous taxon, readily differentiated from allied taxa. Another aim was to try and resolve the taxonomic status of the *Mycobacterium rhodochrous* complex which to date has proved resistant to taxonomic analyses. In addition, it might be possible to ascertain the range of variation within clusters and the quantitative relationships between them. In this way heterogeneous clusters would be spotlighted and provide the subject matter for further specialized studies. Finally, representatives could be chosen rationally to represent defined clusters for use in experiments too lengthy for routine work with many strains.

METHODS

Strains

Nearly all of the 283 organisms were received from public or private culture collections. They included the readily available type or neotype strains of the genus *Nocardia*. In addition to representatives of the better-known species, saprophytic strains of nocardiae isolated from a variety of habitats were studied. Details of the source, past histories, culture collection numbers, etc., of all these strains are given in the results section (Tables 3 to 13). The nocardioform strains carried nine generic epithets. The *Nocardia* strains were received under 37 specific names. On arrival each strain was given a serial number ($N1 \rightarrow Nn$), and the name under which it was received retained.

Initially all the organisms were examined for purity and for their ability to grow on yeast extract agar (YEA). This basal medium proved to be suitable and all strains were maintained on YEA slopes at room temperature, at 4° and at -20°. For long-term storage the organisms were lyophilized.

Collection of data

For each strain 241 features were studied. All test media were inoculated from 14-day-old cultures grown at 30° on YEA or in yeast extract broth. Unless otherwise stated test media were incubated at 30° for 14 days. Tests were generally carried out once, but always repeated when inconclusive results were obtained. Many of the tests were performed in divided polystyrene Replidishes. Each dish is divided into 25 separate compartments and can be readily inoculated by means of a simple multipoint inoculator (Sneath & Stevens, 1967). Tests which required prolonged incubation, or entailed production of gaseous or volatile compounds or the measurement of the zones of clearing of insoluble compounds were performed in tubes or Petri dishes. The 204 unit characters scored for computation are listed in Table 1. The tests are described below.

Table 1. *Unit characters for which all organisms were examined*

Character	No. of states	Character	No. of states
Presence of		Hydrolysis of	
Mycelium	2	Tween 20	2
Permanent mycelium	2	Tween 40	2
Rods and cocci	2	Tween 60	2
Only rods	2	Tween 80	2
Only cocci	2	Tyrosine*	3
Aerial hyphae*	3	Xanthine*	3
Diffusible exopigment	2	Xylan	2
Acid-fast reaction*	3	Production of	
Colony		Allantoinase*	3
Margin	3	Catalase, strong	2
Elevation	3	<i>m</i> -Nitrophenoloxidase	2
Pigmentation	5	<i>p</i> -Nitrophenoloxidase	2
Easy to emulsify	2	Oxidase	2
Suspension coarse	2	Urease*	3
Production of acid from		Reduction of nitrate	2
L-Arabinose	2	Benzidine test*	3
D-Arabinose	2	Utilization, as sole carbon source, of	
D-Xylose	2	The sugars, sugar alcohols	52
L-Rhamnose	2	Starch	2
D-Fructose	2	Glucosamine	2
D-Galactose	2	Sodium acetate	2
D-Glucose (aerobically)	2	Sodium- <i>n</i> -butyrate	2
Mannose	2	Sodium propionate	2
Cellobiose	2	Sodium- <i>n</i> -octoate	2
Lactose	2	Adipic acid	2
Sucrose	2	Pimelic acid	2
Maltose	2	Sebacic acid	2
Trehalose	2	Sodium succinate	2
Melezitose	2	Sodium- <i>H</i> -malate	2
Raffinose	2	Sodium malonate	2
Glycogen	2	Sodium benzoate	2
Inulin	2	Testosterone	2
Amygdalin	2	<i>m</i> -Hydroxybenzoic acid	2
Arbutin	2	<i>p</i> -Hydroxybenzoic acid	2
Salicin	2	L- α -Alanine	2
Adonitol	2	D- α -Alanine	2
Dulcitol	2	DL-nor-Leucine	2
Glycerol	2	L-Threonine	2
Mannitol	2	L-Tryptophan	2
Sorbitol	2	L-Tyrosine	2
Inositol	2	L-Proline	2
Glucose (anaerobically)	2	Betaine HCl	2
Ethanol	2	Acetamide	2
Dextrin	2	Sodium lactate	2
Hydrolysis of		Sodium tartrate	2
Adenine*	3	Sodium citrate	2
Aesculin	2	Sodium pyruvate	2
Casein*	3	Benzamide	2
Cellulose	2	Sodium hippurate	2
Chitin	2	Sodium gluconate	2
DNA	2	Protocatechuic acid	2
Gelatin*	3	L-Serine	2
Guanine	2	<i>p</i> -Cresol	2
Hippurate	2	<i>o</i> -Hydroxybenzaldehyde	2
Hypoxanthine*	3	D-Mandelic acid	2
Keratin*	3	Liquid paraffin	2

Table 1 (*cont.*)

Character	No. of states	Character	No. of states
Utilization, as sole carbon and nitrogen source, of		Resistance to	
Acetamide	2	Phenyl ethanol*	5
Serine	2	Potassium tellurite*	5
Trimethyldiamine	2	Sodium azide*	5
Resistance to		Sodium chloride*	5
Lysozyme	2	Teepol*	4
Penicillin	2	Tetrazolium*	3
Bismuth citrate*	4	Thallous acetate*	4
Crystal violet*	5	Thermotolerance*	6
Phenol*	4	Acid tolerance*	6

* Multistate quantitative character.

Bacterium morphology and staining properties. Smears from YEA slopes showing good growth were prepared, Gram-stained by means of Hucker's modification (Society of American Bacteriologists, 1957), and examined for the presence of a mycelium, fragmentation, pleomorphism, etc. On arrival all strains were stained by a modification of the Ziehl-Neelson method (Gordon, 1967) and the degree of acid-fastness noted. Motility was detected in hanging drop preparations, and in Craigie tubes containing sloppy YEA (Society of American Bacteriologists, 1957).

Colony characters. The morphology of the hyphae and their fragmentation were observed by means of a Watson Stereozoom microscope at $\times 50$ after 1, 3 and 7 days growth on YEA plates. The same plates were examined after 7 and 14 days for colonial features, pigmentation, detection of water-soluble exopigments, aerial hyphae, etc. With butyrous or 'soft' growing nocardioform organisms aerial hyphae production tended to be sparse and could be seen only by focusing on the colony surface. Finally the texture of the colony and ease with which it could be emulsified were recorded.

Fermentation studies. The basal medium of Juhasz & Bönicke (1965) was amended with 0.03% (w/v) bromothymol blue. The concentration of the indicator was less than that recommended by Hugh & Leifson (1953) but did not inhibit the growth of any strains. Inoculated tubes were incubated for three weeks and acid production detected by a change in colour of the medium. With glucose the test was done in duplicate, one tube being sealed with a 1 cm. layer of vaspar. Sterile sugars and sugar alcohols were added to the basal medium to give a final concentration of 1% (w/v). Dulcitol, dextrin and inulin were steamed for 1 h. on three successive days. The remaining carbohydrates were sterilized by filtration. The carbohydrates used are tabulated in Table 1.

Hydrolysis of organic compounds. The hydrolysis of adenine, xanthine, hypoxanthine, tyrosine, guanine (0.2%, w/v), keratin (0.2%, w/v) and xylan (0.4%, w/v) was detected in the basal medium described by Gordon (1967), and chitin degradation in the medium devised by Veldkamp (1955). The plates were heavily inoculated and observed for the disappearance of the insoluble compounds. Zones of clearing greater than 4 mm. were scored as strong positives. Guanine and chitin plates were read weekly for five weeks, the rest for three weeks. The hydrolysis of casein and gelatin was examined by means of the medium and method described by Gordon (1967). The production of deoxyribonucleases was detected in YEA amended with 0.2% (w/v) deoxyribonucleic acid, by means of the method of Jeffries, Holtman & Guse (1957). Hippurate hydrolysis was studied in tubes containing the

medium recommended by Gordon & Horan (1968). After six weeks' incubation a positive result was indicated by the appearance of benzoic acid crystals after an equal volume of 50% sulphuric acid had been added to the medium. Degradation of cellulose strips in YE broth was also observed after six weeks incubation. Tweens 20, 40, 60 and 80 were incorporated into the medium recommended by Sierra (1957) and the media examined for the production of extracellular lipases. Opacity around the areas of growth due to the precipitation of calcium salts was recorded as a positive result. These tests and the detection of aesculin hydrolysis (Sneath, 1966) were carried out in Replidishes.

Organic compounds as sole sources of carbon and energy for growth. Preliminary experiments were designed to find a suitable basal medium for these nutritional studies. Three Replidishes of each of the eight basal media were prepared, one amended with 1% (w/v) glucose and a second with 0.1% (w/v) sodium citrate. The third dish was the control and contained no carbon source. The basal media tested were those of Pridham & Gottlieb (1948), McClung (1954), Gordon & Mihm (1957), Jones & Bradley (1964), Juhasz & Bönické (1965), Stanier, Palleroni & Doudoroff (1966), Stevenson (1967) and Jones & Edington (1968). The prepared dishes were inoculated with 25 randomly selected nocardioform organisms. Only two of the media consistently supported good growth of the test strains together with negligible background growth on control dishes. The basal medium of Stanier *et al.* (1966) was rejected in favour of that of Stevenson (1967), which is much easier to prepare.

The selected basal medium contained 'Bacto Yeast Nitrogen Base w/o amino acids' (Difco Laboratories) to which Ionagar and 10.0 mg. Casamino acids/l. were added. Before this mixture was dispensed 800 ml. amounts were neutralized with 200 ml. sterile 1% (w/v) K_2HPO_4 . Most of the organic salts were sterilized separately by autoclaving and added to the basal medium at the appropriate concentration. If the thermostability of a compound was in doubt, sterilization was by filtration. The sugars and sugar alcohols were sterilized as before.

The inoculated Replidishes were scored visually after 7 and 14 days. There were two control plates; one contained the basal medium without any carbon source, the second basal medium plus 0.1% (w/v) glucose. A positive result was registered if growth in the test dish was greater than in the control dish without the carbon source, and negative where growth was equal to or less than that on the control plate. Spurious negative results were spotted when test plates were compared with control plates containing glucose. Inconclusive results were rare, provisionally scored $\pm$, and repeated. For the first 40 tests duplicate dishes were prepared but the results were in such good agreement that this practice was discontinued.

The concentration of the different carbon sources in the basal medium varied. The final concentration was intended to be sufficient to avoid substrate exhaustion but not enough to inhibit the growth of any of the strains. The 102 high-purity compounds listed below were examined as sources of carbon and energy for growth.

(a) Carbohydrates and sugar derivatives (1%, w/v): L-arabinose, D-arabinose, D-xylose, L-rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, cellobiose, lactose, sucrose, maltose, trehalose, melezitose, raffinose, glycogen, inulin, amygdalin, arbutin, salicin, dextrin, starch, glucosamine, gluconate.

(b) Polyalcohols and glycols (1%, w/v): adonitol, dulcitol, glycerol, mannitol, *meso*-inositol, sorbitol.

(c) Fatty acids (0.1%, w/v): sodium formate, acetate, propionate, *n*-butyrate, *n*-octoate, *H*-malate, pelargonic acid.

(d) Dicarboxylic acids (0.1 %, w/v): 1, saturated: sodium oxalate, malonate, succinate, adipic acid, pimelic acid, sebacic acid; 2, unsaturated: sodium fumarate.

(e) Aliphatic amino acids (0.1 %, w/v): D- α -alanine, L- α -alanine, DL-arginine HCl, DL-nor-leucine, L-threonine, DL-ornithine, L-serine.

(f) Amino acids with a ring structure (0.1 %, w/v): L-tryptophan, D-tryptophan, L-proline, L-tyrosine.

(g) Amines (0.1 %, w/v): histamine, putrescine.

(h) Hydroxyacids (0.1 %, w/v): sodium citrate, lactate, D-tartrate.

(i) Aromatic acids, 0.1 % (w/v): sodium hippurate, *o*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, D-mandelic acid, L-mandelic acid, *o*-nitrobenzoic acid, phenylglyoxylic acid, protocatechuic acid, shikimic acid; 0.05 % (w/v): anthranilic acid, genistic acid, nicotinic acid, phthalic acid, syringic acid, vanillic acid; 0.01 % (w/v): quinic acid.

(j) Phenols and phenolic derivatives and homologues, 0.025 % (w/v): catechol, *o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol; 0.05 % (w/v): *p*-cresol.

(k) Steroids (0.1 %, w/v): indole-3-acetic acid, testosterone.

(l) Polynuclear aromatics (0.1 %, w/v): naphthalene.

(m) Monobasic acid with the carboxyl group in the side-chain (0.025 %, w/v): cinnamic acid.

(n) Aromatic aldehydes, 0.025 % (w/v): syringaldehyde; 0.05 % (w/v): vanillin.

(o) Keto acids (0.1 %, w/v): sodium pyruvate.

(p) Miscellaneous non-nitrogenous aromatic compounds, 0.1 % (w/v): sodium alginate benzoyl-formate, benzoate, mucic acid; 0.05 % (w/v): ferulic acid, *o*-hydroxybenzaldehyde, *p*-hydroxybenzaldehyde; 0.025 % (w/v): coumarin.

(q) Miscellaneous nitrogen compounds (0.1 %, w/v): acetamide, benzamide, benzylamine, betaine HCl.

(r) Paraffin hydrocarbons (1 %, w/v): liquid paraffin.

Organic compounds as sole sources of carbon and nitrogen for growth. The three compounds tested – acetamide, serine and trimethyldiamine – were added to the basal medium described by Tsukamura (1966).

Tolerance to chemical inhibitors, temperature and pH. In all these tests the basal medium was YEA. For the inhibition tests various concentrations of sodium azide, sodium chloride, bismuth citrate, crystal violet, phenol, potassium tellurite, phenyl ethanol, Teepol, tetrazolium and thallos acetate were added to the basal medium. The ability of strains to grow at 10°, 35°, 40°, 45° and 50°, and in media of pH 4 to pH 10, was examined. All these tests were carried out in Replidishes. After 14 days' incubation tests were scored as positive or negative, depending upon the presence or absence of growth. The sensitivity of strains to 5 i.u. of penicillin was examined with antibiotic discs. This test was carried out in plates and read after 7 days.

Biochemical tests. Catalase, oxidase (Steel, 1961) and cytochrome oxidase (Deibel & Evans, 1960) production was examined on 1- to 5-day YEA cultures. The medium described by Tacquet (1962) was used to detect the formation of *m*-nitrophenoloxidase and *p*-nitrophenoloxidase. Allantoinase, nitrataase and urease activity were detected by means of the media and methods of Gordon (1967), the results being recorded weekly for 4 weeks. Resistance to lysozyme was determined according to the method described by Gordon (1966a).

Coding of features for computation

Most features existed in one of two mutually exclusive states, and were scored for computation by recording positive reactions as 1 and negative as 0. Pigmentation and colonial characters were divided into 2 to 5 mutually exclusive states; organisms were coded 1 for the particular character state they exhibited and 0 for the others. The additive method of Sokal & Sneath (1963) was used to code multistate quantitative characters, tolerance to chemical inhibitors, pH and temperature, some of the hydrolysis tests and for the benzidine test. Inapplicable characters, such as the detection of pleomorphism in strains with a permanent mycelium, were designated 10₉ (non comparable) and ignored in calculating similarity coefficients. Thirty-seven characters gave either all positive or all negative results and as they had no discriminating value they were not included for computation (Table 2). Each strain was therefore tested for 204 unit characters (Table 1). All the results were entered onto standard IBM punch cards.

Table 2. *Characters which have no discriminating value*

Utilization, as sole carbon source, of:	
Sodium alginate	<i>o</i> -Nitrophenol
Sodium formate	<i>m</i> -Nitrophenol
Sodium fumarate	<i>p</i> -Nitrophenol
Sodium oxalate	Indole-3 acetic acid
Pelargonic acid	Naphthalene
<i>o</i> -Hydroxybenzoic acid	Cinnamic acid
DL-Arginine HCl	Syringaldehyde
DL-Ornithine	Vanillin
D-Tryptophan	Benzoylformate
Histamine	Mucic acid
Putrescine	Ferulic acid
L-Mandelic acid	<i>p</i> -Hydroxybenzaldehyde
Anthranilic acid	Coumarin
Genistic acid	
Nicotinic acid	Resistance to
Phthalic acid	15 % NaCl
Syringic acid	pH 4
Vanillic acid	
Quinic acid	Biochemical
Catechol	Catalase, weak

Computation

Because of the large amount of data, computation was carried out in two stages. In the first analysis the pathogenic strains, *Nocardia asteroides*, *N. brasiliensis*, *N. caviae*, *N. madurae* and *N. pelletieri*, were considered, and in the second the soft-growing nocardioform organisms. The overall similarity between strains was calculated in two ways – by the method devised by Sneath (1957) in which negative similarities are excluded, and by the method of Sokal & Michener (1958) in which negative similarities are included. Thus, where *S* is the similarity coefficient (usually expressed as a percentage), *n_{sp}* and *n_{sn}* the number of positive and negative similarities respectively, and *n_a* the number of non-matching characters, then according to Sneath;

$$S = n_{sp}/(n_{sp} + n_a),$$

and according to Sokal and Michener,

$$S = (n_{sp} + n_{sn})/(n_{sp} + n_{sn} + n_a).$$

The organisms in each of the four unsorted similarity matrices so obtained were then rearranged into significant groups. Cluster analysis was carried out using two techniques: the

single linkage method (Sneath, 1957) and the unweighted average linkage method (Sokal & Michener, 1958). All the calculations were carried out on an Elliot computer at Leicester University.

RESULTS AND DISCUSSION

Formation of clusters

Eighty-five per cent of 239 test strains could be assigned to one of 15 clusters. The quantitative taxonomic relationships within and between the clusters are graphically illustrated in the sorted similarity matrices (Fig. 1, 2). Dendrograms derived from the matrices, and based on unweighted average linkage cluster analysis, are shown in Fig. 3 to 6. Nine major and six small minor clusters could be recognized at the 80% Similarity Level (S-level). For the sake of clarity, and where it was possible, the clusters were given the name most frequently carried by isolates within a cluster. Seven of the major clusters could thus be roughly equated with the species: *Nocardia asteroides*, *N. caviae*, *N. brasiliensis*, *N. madurae*, *N. pelletieri*, *N. turbata* and *Mycobacterium rhodochrous*. There were too few strains in the minor clusters for them to be designated in this way.

Cluster 1. The *Nocardia asteroides* cluster. Taxonomically this was one of the more complex clusters where the 42 strains fell into one of the five subgroups (Table 3). The subgroups were defined at the 85% S-level or above. Over half the organisms were received as *N. asteroides*, and the others carried specific epithets often considered to be synonyms of this species. Subgroups 1A, 1B and 1C contain strains which shared a high overall similarity. The most homogeneous subgroup comprises the six strains of *N. farcinica* which coalesced above the 90% S-level. Included in subgroup 1C is strain N34 (ATCC 3318), the type species of the genus. Gordon & Mihm (1957) considered that the names *N. asteroides* and *N. farcinica* referred to a single group, and proposed that the type species *N. farcinica* be accepted as a synonym of *N. asteroides*. In contrast Tsukamura (1969) found two major clusters of *N. asteroides* and redesignated the one which contained the type species *N. farcinica*. The strains *N. asteroides* (N76 and N79) and *N. blackwellii* (N122) were included by Tsukamura in the cluster *N. farcinica*. In the present study subgroup 1C contains the type species, is distinct from the other subgroups, but includes none of the other common strains found in Tsukamura's *N. farcinica* cluster. *Nocardia asteroides* (N76) is in subgroup 1E, the remaining two strains in subgroup 1A. Again two strains of *N. asteroides* (N131 and N140) are found in subgroups 1A and 1B respectively. Tsukamura (1969) allotted them to a single cluster, *N. asteroides* group Kyoto-11. He also placed in this cluster *N. corallina* (N84), which was found by me to be a typical strain of *Mycobacterium rhodochrous*.

Subgroups 1D and 1E are small and relatively heterogeneous. All the *Nocardia paraffinae* strains fell into subgroup 1D, which also contains the suggested working-type strain of *N. asteroides*, N317 (Sneath & Skerman, 1966). Subgroup 1E is only marginally included in cluster 1.

Cluster 2. The *Nocardia caviae* cluster. Ten strains fell into one or other of the two subgroups (Table 4). Each subgroup is homogeneous and defined at or around the 90% S-level.

Cluster 3. This cluster contains eight strains (Table 5), is diffuse and, as it stands, of little taxonomic value. A high phenetic similarity is shared, however, by the two strains of *Nocardia rhodnii* and also by *N. convoluta* (N95) and *N. pasteuroides*. These two minor subgroupings may form nuclei around which additional strains may cluster.

Cluster 5. The *Nocardia brasiliensis* cluster. Ten strains joined above the 85% S-level to form a tight homogeneous group (Table 6). This cluster contains the working-type strain of

Fig. 3. Dendrogram of the relationships within and between clusters 1 to 6, negative matches included.

Fig. 1. A sorted similarity matrix of the 'hard' growing nocardioform strains.

Fig. 1. A sorted similarity matrix of the 'hard' growing nocardioform strains.

Fig. 4. Dendrogram of the relationships within and between clusters 7 to 13, negative matches included.

Fig. 5. Dendrogram of the relationships with the *M. rhodochrous* cluster, negative matches included.

Fig. 6. Dendrogram of the relationships within cluster 15 and between the unplaced strains.

N. brasiliensis and corresponds to the recognizable species bearing that specific name. *Nocardia pretoriana* (N237) is loosely associated with, but not included in, this cluster.

Cluster 7. The *Nocardia madurae* cluster. Although all 22 strains included in this cluster (Table 7) were received as either *N. madurae* or *Streptomyces madurae*, the cluster is very diffuse. Thus while the strains were divided into five subgroups, no two strains shared a

Table 3. Strains assigned to cluster 1, the *Nocardia asteroides* group

Subgroup 1 A	
N13	<i>N. asteroides</i> (Eppinger) Blanchard; NCTC 8595, strain Greece-Salonika; ATCC 14759. Isolated by R. H. Gorrill from pus of empyema (<i>Nocardia sebiivorans</i>)
N96	<i>N. asteroides</i> ; Dr R. E. Gordon, Rutgers University, New Brunswick, U.S.A., w 3300; S. A. Waksman; A. T. Henrici
N97	<i>N. asteroides</i> ; Dr R. E. Gordon, N659 (<i>N. caprae</i>)
N98	<i>N. asteroides</i> ; Dr R. E. Gordon, 618; E. N. Azarowicz, 35; CBS 242.37; Polacci
N99	<i>N. asteroides</i> ; Dr R. E. Gordon, 508; received from P. Thibault as <i>N. eppingerii</i> . Isolated from brain abscess
N100	<i>N. asteroides</i> ; Dr R. E. Gordon, 652 (<i>N. phenotolerans</i>); E. N. Azarowicz, 110; A. Gonz��les Ochoa, 1139; A. L. Carri��n
N102	<i>N. asteroides</i> ; Dr R. E. Gordon, 653; E. N. Azarowicz, 111; A. Gonz��les Ochoa, 1028; D. Erikson. Type strain of <i>N. rhodnii</i>
N105	<i>N. asteroides</i> ; Dr R. E. Gordon, 9969; ATCC 9969; C. W. Emmons, 9955; P. Hadley. Isolated from generalized actinomycosis
N106	<i>N. asteroides</i> ; Dr R. E. Gordon, A9504; ATCC 9504. Isolated by O. Felsenfeld from Madura foot
N131	<i>N. asteroides</i> var. <i>crateriforme</i> ; CBS 242.37; Polacci
N211	<i>N. asteroides</i> ; Dr J. E. Thiemann, Lepetit S.p.A; Milan, Italy, s545; PSA 158; ATCC 9504; R. E. Gordon, A9504. Isolated by O. Felsenfeld from Madura foot
N77	<i>N. blackwellii</i> (Erikson) Waksman & Henrici; Dr S. T. Williams, Hartley Botanical Laboratories, University of Liverpool, England, E15; NCTC 630 (<i>Actinomyces blackwellii</i>); J. McFadyean; J. T. Edwards. Isolated from hock joint of foal. ATCC 6846
N122	<i>N. blackwellii</i> ; CBS 272.53; ATCC 6846
N127	<i>N. caprae</i> (Silberschmidt) Waksman & Henrici; CBS 248.33; Institut Pasteur, Paris
N214	<i>N. caprae</i> ; Dr J. E. Thiemann, s469; PSA 31; CBS
N121	<i>N. leishmanii</i> Chalmers & Christopherson; CBS 333.51; R. Ciferri, 392
N210	<i>N. polychromogenes</i> (Vall��e) Waksman & Henrici; Dr J. E. Thiemann, A4; PSA 36; CBS
N204	<i>N. sylvodorifera</i> Castellani; ATCC 7372; A. Castellani (<i>Arthrobacter sylvodoriferus</i>). Type strain
Subgroup 1 B	
N101	<i>N. asteroides</i> ; Dr R. E. Gordon, w 3410; NCTC (<i>N. paraffinae</i>)
N103	<i>N. asteroides</i> ; Dr R. E. Gordon, A8674 (<i>N. minima</i>); ATCC 8674; NCTC 3489; H. L. Jensen. Type strain of <i>Proactinomyces minimus</i>
N104	<i>N. asteroides</i> ; Dr R. E. Gordon, w 3409A (<i>N. polychromogenes</i>); S. A. Waksman
N225	<i>N. asteroides</i> ; Dr G. L. Bullock, Eastern Fish Disease Lab., Kearneysville, U.S.A., L-10.4. Isolated from fingerling Rainbow Trout (<i>Salmo gairdneri</i>)
N228	<i>N. asteroides</i> ; NCMB 1470; D. A. Conroy. Isolated from neon tetras (<i>Hyphessobrycon innesi</i>) with symptoms of piscine tuberculosis
N233	<i>N. asteroides</i> ; Dr R. Olds, Pathology Department, University of Cambridge, England, CN750. Isolated from milk of cow
N249	<i>N. asteroides</i> subgroup; Dr T. Cross, University of Bradford, England, CUB118; FBA-W28. Isolated from freshwater of Blelham Tarn
N265	<i>N. sp. asteroides</i> type. Dr T. Cross, CUB415; J. Lacey, A747. From air sample while opening hay bales in open
N224	<i>N. leishmanii</i> ; Dr J. E. Thiemann, A33; PSA 27; CBS
N70	<i>N. minima</i> (Jensen) Waksman & Henrici; Institute of Applied Microbiology, University of Tokyo, IAMO374
N216	<i>N. phenotolerans</i> Werkman & Patrick; Dr J. E. Thiemann, s547; PSA 161; IMRU 652; E. N. Azarowicz, 110; A. Gonz��les Ochoa, 1139; A. L. Carri��n (<i>A. phenotolerans</i>)

Table 3 (cont.)

Subgroup 1 C	
N 34	<i>N. farcinica</i> Trevisan; NCTC 4524. Cotype (Sneath & Skerman, 1966)
N 120	<i>N. farcinica</i> ; CBS 223.60; ATCC 13781. Isolated by Dr Mornet, from a case of bovine farcy in Senegal
N 307, 308	<i>N. farcinica</i> ; Professor F. Mariat, Institut Pasteur, Paris, 738, 735. Isolated from cases of bovine farcy in Chad
N 333, 334	<i>N. farcinica</i> ; Professor H. A. Lechevalier, Rutgers University, New Brunswick, U.S.A. 1223, 1225
Subgroup 1 D	
N 317	<i>N. asteroides</i> ; ATCC 19247; R. E. Gordon, 727; L. Ajello, M 170-6; W. M. Bowman. Suggested working type (Sneath & Skerman, 1966)
N 119	<i>N. paraffinae</i> (Jensen) Waksman & Henrici; CBS 255.58; Dr Borghans, Willemstad, 13482. Isolated from a lesion of an ear
N 142	<i>N. paraffinae</i> ; Department of Agriculture, University of Sydney, 2171
N 201	<i>N. paraffinae</i> ; CBS 514.67. Isolated from plaster work
N 72	<i>N. polychromogenes</i> ; International Center for the Distribution of Type Cultures, Lausanne, Switzerland. LA 1610
Subgroup 1 E	
N 71	<i>N. asteroides</i> ; LA 1613; Institut Pasteur, Paris, 301
N 76	<i>N. asteroides</i> ; Dr S. T. Williams, E 13; NCTC 6761

Table 4. Strains assigned to cluster 2, the *Nocardia caviae* group

Subgroup 2 A	
N 130	<i>N. asteroides</i> ; CBS 226.38. Received as <i>N. madurae</i> by Pinoy.
N 3	<i>N. uniformis</i> Marton & Szabo; NCIB 9631; L. Silvestri
N 129	<i>N. uniformis</i> ; CBS 224.60; I Szabo
N 200	<i>N. uniformis</i> ; Dr I Szabo, Laboratory of Soil Biology, Sopron, Hungary, 1849
Subgroup 2 B	
N 21	<i>Proactinomyces caviae</i> Snijders; CCM 197; J. de Ley
N 36	<i>N. caviae</i> (Erikson) Gordon & Mihm; NCTC 1934; E. P. Snijders; ATCC 14629. Isolated from an infected ear of a guinea pig. Cotype (Sneath & Skerman, 1966)
N 231	<i>N. caviae</i> ; Dr R. Olds, CN 749. Isolated from a dachshund
N 232	<i>N. caviae</i> ; Dr R. Olds, CN 751. Isolated from a corgi
N 313, 314	<i>N. caviae</i> ; Professor F. Mariat, 751, 771

Table 5. Strains assigned to cluster 3

N 208	<i>Nocardia asteroides</i> ; Dr J. E. Thiemann, A 8; PSA 33; CBS
N 15	<i>N. congolensis</i> ; NCTC 5175
N 95	<i>N. convoluta</i> Chalmers & Christopherson; Dr A. Gonz��les Ochoa, Instituto de Salubridad y Enfermedades Tropicales, Mexico 17, DF 98
N 140	<i>N. convoluta</i> ; Dr J. Antheunisse, Landbouwhogeschool, Wageningen, Holland; ATCC 4275; H. J. Conn (<i>Mycobacterium convolutum</i>); P. H. H. Gray
N 94	<i>N. pasteuroides</i> ; Dr A. Gonz��les Ochoa, 35
N 219, 220	<i>N. rhodnii</i> (Erikson) Waksman & Henrici; Professor C. da Silva Lacaz, Universidade de S��o Paulo, Brazil. Isolated from the gut of <i>Rhodnius prolixus</i>
N 217	<i>N. sylvodorifera</i> Castellani; Dr J. E. Thiemann, S 546; PSA 160; ATCC 7372, A. Castellani. Type strain

high phenetic similarity. The suggested working-type strain of the species (Sneath & Skerman, 1966) is found in subgroup 7 A.

Cluster 8. The Nocardia pelletieri cluster. The strains are listed in Table 8. They fell into two contrasting subgroups linked together just above the 80% S-level. With a single

exception the eleven strains received as either *N. pelletieri* or *Streptomyces pelletieri* coalesced at or above the 85% S-level. The three strains of *Dermatophilus congolensis* form the centre of the second tight subgroup. The species *Geodermatophilus obscuratus* is included at the periphery of this subgroup. The association of the subgroups within a single cluster is probably more apparent than real.

Table 6. *Strains assigned to cluster 5, the Nocardia brasiliensis group*

N14	<i>N. brasiliensis</i> (Lindenberg) Pinoy; NCTC 10300; ATCC 19295
N48	<i>N. brasiliensis</i> ; Dr R. E. Gordon, 744; A. Gonz��les Ochoa, 409; ATCC 91295
N107	<i>N. brasiliensis</i> ; Dr R. E. Gordon, N2391 (<i>Nocardia pretoriana</i>); NCTC 2391
N118	<i>N. brasiliensis</i> ; CBS 438.64; A. H. Klokke, 300. Isolated from multiple sinus in right knee
N212	<i>N. brasiliensis</i> ; Dr J. E. Thiemann, S580; PSA 213; IMRU 774
N213	<i>N. brasiliensis</i> ; Dr J. E. Thiemann, S581; PSA 214; IMRU 848; J. D. Schneidau, Jun. 386B; C. da Silva Lacaz, 85
N218	<i>N. brasiliensis</i> ; Professor C. da Silva Lacaz
N311	<i>N. brasiliensis</i> ; Professor F. Mariat, 768
N312	<i>N. brasiliensis</i> ; Professor F. Mariat, 337
N318	<i>N. brasiliensis</i> ; ATCC 19296; R. E. Gordon, 845; J. D. Schneidau, Jun., 381; A. Batista, 631; Institut Pasteur, Paris, 337. Uncertain cotype (Sneath & Skerman, 1966)

Table 7. *Strains assigned to cluster 7, the Nocardia madurae group*

Subgroup 7A	
N17	<i>N. madurae</i> (Vincent) Blanchard; NCTC 1070. Isolated from a case of Madura foot
N35	<i>N. madurae</i> ; NCTC 5654; ATCC 19425. Isolated by J. T. Duncan from mycetoma of foot. Suggested working type (Sneath & Skerman, 1966)
N80	<i>N. madurae</i> ; Dr S. T. Williams, E23; R. E. Gordon, 507; P. Thibault
N81	<i>N. madurae</i> ; Dr S. T. Williams, E24; R. E. Gordon, 431; I. Christison; N. F. Conant, 2177; A. Gonz��les Ochoa, 415
N263	<i>N. madurae</i> type; Dr T. Cross, CUB 410; J. Lacey. Isolated from stored hay
N283, 284	<i>Streptomyces madurae</i> ; Professor I. G. Murray, School of Tropical Medicine, London, 1155; 759
N286	<i>S. madurae</i> ; Professor I. G. Murray, 1056
Subgroup 7B	
N252	<i>N. madurae</i> ; Dr T. Cross, CUB 130; J. Lacey; M. P. Lechevalier. Isolated from soil
N269, 270	<i>N. sp. madurae</i> type; Dr T. Cross, CUB 436, 437; J. Lacey A 471, A 683. Isolated from moist barley grain
Subgroup 7C	
N303	<i>S. madurae</i> ; Professor F. Mariat, 364. Isolated from a case of Madura foot
N304	<i>S. madurae</i> ; Professor F. Mariat, 391
N305	<i>S. madurae</i> ; Professor F. Mariat, 373. From a case of mycetoma, Fort Lamy, Chad
N306	<i>S. madurae</i> ; Professor F. Mariat, 767
N316	<i>S. madurae</i> ; Professor F. Mariat, 384
Subgroup 7D	
N251	<i>N. madurae</i> subgroup; Dr T. Cross, CUB 123; P. C. K. Austwick, v6973C
N266, 267	<i>N. sp. madurae</i> type; Dr T. Cross, CUB 433-4; J. Lacey, A 151, 322. Isolated from sugar cane bagasse and hay respectively
Subgroup 7E	
N250	<i>N. madurae</i> subgroup; Dr T. Cross, CUB 122; P. C. K. Austwick, v673B (<i>S. fradiae</i>)
N268	<i>N. sp. madurae</i> type; Dr T. Cross, 435; J. Lacey, A 337. Isolated from oat grain
N285	<i>S. madurae</i> ; I. G. Murray, 767

Table 8. Strains assigned to cluster 8, the *Nocardia pelletieri* group

Subgroup 8 A	
N 18	<i>N. pelletieri</i> (Laveran) Pinoy; NCTC 10000
N 49	<i>N. pelletieri</i> ; Dr R. E. Gordon, 513; P. Thibault
N 78	<i>N. pelletieri</i> ; Dr S. T. Williams, E 21; R. E. Gordon, 408; J. M. Coffey, 47293
N 79	<i>N. pelletieri</i> ; Dr S. T. Williams, E 22; R. E. Gordon, 610; E. N. Azarowicz, 10
N 281, 282	<i>Streptomyces pelletieri</i> ; Professor I. G. Murray, 1065, 1067
N 298, 299	<i>S. pelletieri</i> ; Professor F. Mariat, 726, 381
N 300, 302	<i>S. pelletieri</i> ; Professor F. Mariat, 385, 394, 729
Subgroup 8 B	
N 114 to 116	<i>Dermatophilus congolensis</i> van Saceghem; Professor V. B. D. Skerman, University of Queensland, Australia, 530-2
N 356	<i>Geodermatophilus obscuratus</i> Luedemann; Dr G. M. Luedemann, Schering Corporation, Bloomfield, New Jersey, D 20. Isolated from soil

Table 9. Strains assigned to cluster 10, the *Nocardia turbata* group

Subgroup 10 A	
N 32	<i>N. turbata</i> Erikson; Dr H. Lautrop, Statens Seruminstitut, Copenhagen, Denmark, AB 1476; J. Ørskov, 27
N 50	<i>N. turbata</i> ; Dr D. M. Webley, Macaulay Institute, Aberdeen, Scotland, strain C; R. E. Gordon, 763
N 51	<i>N. turbata</i> ; Dr D. M. Webley, strain B; R. E. Gordon, 762
N 52	<i>N. turbata</i> ; Dr D. M. Webley, strain A ₃ ; R. E. Gordon, 761
N 40	<i>N. cellulans</i> Metcalf and Brown; NCIB 8868; M. E. Brown, ATCC 12830. Cotype (Sneath and Skerman, 1966). Isolated from soil
Subgroup 10 B	
N 41	<i>N. calcarea</i> Metcalf and Brown; NCIB 8863; ATCC 19369. Cotype (Sneath & Skerman, 1966). Isolated from soil, M. E. Brown
N 144, 145	<i>N. marina</i> (Krassilnikov) Waksman; Mrs M. Turner, University of Nottingham, England; 35-6. Isolated from decaying seaweed.

Cluster 10. The *Nocardia turbata* cluster. The strains in this cluster fell into two distinct and contrasting subgroups (Table 9). The four *N. turbata* strains unite above the 95 % S-level to form a tight centre to subgroup 10 A. The type strain of *N. cellulans* is found at the periphery of this subgroup. Two strains of *N. marina* and the type strain of *N. calcarea* form the second fragmented subgroup.

Cluster 14. The *Mycobacterium rhodochrous* cluster. The 104 organisms in this cluster are assigned to subgroups (Table 10).

Homogeneous subgroups. Four of the subgroups contain strains which share a relatively high overall similarity. Subgroup 14 A is formed by *Nocardia pellegrino* strains fused together above the 95 % S-level. Two strains of *Mycobacterium rhodochrous* uniting just below the 90 % S-level make the second subgroup. Strains bearing this name are also found in three of the other subgroups. Subgroup 14 C contains 19 strains linked above the 90 % S-level. It contains eight *M. rhodochrous* strains and organisms carrying seven specific epithets attached to either *Nocardia* or *Proactinomyces*. Twenty-four strains are assigned to the largest subgroup, 14 D, also delineated above the 90 % S-level. Almost half were received as *M. rhodochrous*, the additional ones as eight species attributed to the genus *Jensenia*, *Nocardia* or *Proactinomyces*.

Table 10. *Strains assigned to cluster 14, the Mycobacterium rhodochrous group*

Subgroup 14A	
N324	<i>Nocardia pellegrino</i> Pellegrino; ATCC 15998; G. Castelnuova; Institut Pasteur; L. Pellegrino
N325 to 8	<i>N. pellegrino</i> ; Professor G. Castelnuova, Istituto Superiore Di Sanita, Rome, 906B, 107, 906, 350
Subgroup 14B	
N30	<i>Mycobacterium rhodochrous</i> (Zopf) Gordon & Mihm; Dr R. E. Gordon, A12974 (<i>Corynebacterium fascians</i>); ATCC 12974; W. H. Burkholder, CF17; P. E. Tilford
N31	<i>M. rhodochrous</i> ; Dr R. E. Gordon, w21 (<i>Micrococcus rhodochrous</i>); S. A. Waksman; R. S. Breed
Subgroup 14C	
N54	<i>M. rhodochrous</i> ; ATCC 13808; R. E. Gordon, 372 (<i>Rhodococcus rhodochrous</i>); R. S. Breed; Král collection
N55	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 817 (<i>Mycobacterium</i> sp.); N. M. McClung, B161A
N61	<i>M. rhodochrous</i> ; Dr R. E. Gordon, w3408 (<i>N. corallina</i>); S. A. Waksman; ATCC 4273; H. J. Conn; P. H. H. Gray, 0-3
N67	<i>M. rhodochrous</i> ; NCTC 10210
N110	<i>M. rhodochrous</i> ; Dr R. E. Gordon, w3639 (<i>N. rubra</i>); S. A. Waksman; G. Giolitti
N111	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 562 (<i>Proactinomyces ruber</i>); N. M. McClung; CBS
N239	<i>M. rhodochrous</i> ; NCTC 8727
N240	<i>M. rhodochrous</i> ; NCTC 8571
N84	<i>N. corallina</i> ; (Berger <i>et al.</i>) Waksman & Henrici; Dr S. T. Williams, E41; CBS 197.57; ATCC 4273
N73	<i>N. rubra</i> (Kruse) Chalmers & Christopherson; Dr S. T. Williams, E40; CBS
N75	<i>N. rubra</i> ; LA 1609
N83	<i>N. rubra</i> ; Dr S. T. Williams, E40; CBS
N5	<i>N. salmonicolor</i> (den Dooren de Jong) Waksman & Henrici; NCIB 9701
N112	<i>N. salmonicolor</i> ; Professor V. B. D. Skerman, 121
N128	<i>N. salmonicolor</i> ; CBS 196.57
N22	<i>P. corallinus</i> (Hefferan) Jensen; Dr M. Kocur, J. E. Purkyně University, Brno, CCM 3245, ATCC 4273 (<i>N. corallina</i>)
N26	<i>P. luteus</i> Christopherson & Archibald; CCM 198; J. de Ley
N27	<i>P. phenoli</i> ; M. Kocur, BS 278; NCIB 9160
N28	<i>P. ruber</i> Krassilnikov; CCM 269
Subgroup 14D	
N53	<i>Jensenia canicruria</i> Bisset & Moore; NCIB 8147; ATCC 11048. Type strain
N143	<i>J. canicruria</i> ; Mrs M. Turner, 29. Type strain
N56	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 1256 (<i>N. restrictus</i>); A. I. Laskin, 2914. Isolated from soil
N57	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 1293s (<i>Arthrobacter</i>)
N58	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 1257 (<i>N. restrictus</i>); A. I. Laskin; E. R. Squibb and Sons, 2915. Isolated from soil
N59	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 1240 (<i>Corynebacterium rubrum</i>); A. J. Crowle
N60	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 1293R (<i>Arthrobacter</i> sp.)
N62	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 768 (<i>P. opacus</i>), D. M. Webley, T14
N63	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 463 (<i>Mycobacterium</i> sp.); T. C. Stadtman
N64	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 417 (<i>N. globerula</i>); W. C. Haynes, B-1306; ATCC 9356
N65	<i>M. rhodochrous</i> ; Dr R. E. Gordon, A7698 (<i>C. equi</i>); ATCC 7698; H. W. Schoening
N108	<i>M. rhodochrous</i> ; Dr R. E. Gordon, A4277 (<i>N. erythropolis</i>); ATCC 4277; H. J. Conn; P. H. H. Gray, 0-5. Type strain
N109	<i>M. rhodochrous</i> ; Dr R. E. Gordon, 494 (<i>P. globerulus</i>); P. H. H. Gray, M47
N148	<i>N. corallina</i> ; Mrs M. Turner, 83. Isolated from soil
N11	<i>N. erythropolis</i> (Gray & Thornton) Waksman & Henrici; NCIB 9158; M. Kocur, BS 277 (<i>P. erythropolis</i>). Cotype (Sneath & Skerman, 1966)
N209	<i>N. erythropolis</i> ; Dr J. E. Thiemann, A2; PSA 68; CBS

Table 10 (cont.)

N12	<i>N. globerula</i> (Gray) Waksman and Henrici; NCIB9159; M. Kocur, BS279 (<i>P. globerulus</i>); ATCC19370. Cotype (Sneath & Skerman, 1966)
N123	<i>N. lutea</i> Christopherson & Archibald; CBS334.51; R. Ciferri, 373
N38	<i>N. opaca</i> (den Dooren de Jong) Waksman & Henrici; NCIB9409; D. M. Webley, strain T16
N4	<i>N. rubropertincta</i> (Hefferan) Waksman and Henrici; NCIB9664
N113	<i>N. rubropertincta</i> ; Professor V. B. D. Skerman, 134
N257	<i>Nocardia</i> spp.; Dr T. Cross, CUB197. Isolated from Blelham Tarn
N23	<i>P. canicruria</i> Jensen; CCM277; P. H. H. Gray; ATCC4277
N25	<i>P. globerulus</i> Reed; CCM279; P. H. H. Gray

Subgroup 14E

N7	<i>N. restrictus</i> Turfitt; NCIB10027
N10	<i>N. aurantia</i> ; NCIB9557; ATCC12674
N66	<i>M. rhodochrous</i> ; NCTC8139
N124, 125	<i>N. opaca</i> ; CBS330.61, 331.61. Isolated by Dooren de Jong, 279, 292. Type strains
N135	' <i>Nocardia</i> sp.' Dr J. Antheunisse, AC338. Isolated from rhizosphere of grass
N137	' <i>Nocardia</i> sp.' Dr J. Antheunisse, AC339. Isolated from rhizosphere of grass
N153	' <i>Arthrobacter</i> sp.' Mrs M. Turner, x45. Isolated from seaweed
N146	<i>C. fascians</i> (Tilford) Downson; Mrs M. Turner, 39
N234	<i>Nocardia</i> sp.; Professor H. Veldkamp, University of Groningen, Holland, 4x
N272	<i>Nocardia</i> sp.; Dr P. Latter, Merlewood Research Station, Grange-over-Sands, England. 19/7 56/4. Isolated from <i>Juncus</i> sp. litter
N274, 275	<i>Nocardia</i> sp.; Dr P. Latter, 30/7 34/2; $\frac{3}{8}$ 36/13. Isolated from soil on Signy Island

Subgroup 14F

N147 to 149	'coryneform'; Mrs M. Turner, 65, 93. Isolated from soil
N150 to 152	'coryneform'; Mrs M. Turner x10, x18, x34. Isolated from seaweed
N154, 155	'arthrobacter'; Mrs M. Turner, x55-6. Isolated from seaweed

Subgroup 14G

N16	<i>N. cuniculi</i> Snijders; NCTC1935 (<i>A. sumatrae</i>)
N346	<i>N. flava</i> (Krassilnikov) Waksman and Henrici; CBS, strain Vrugink
N19	<i>Nocardia</i> sp.; NCTC7915
N37	<i>N. salivae</i> Davis & Freer; NCTC10207. Holotype (Sneath & Skerman, 1966)
N47	<i>Nocardia</i> sp.; Dr M. Goodfellow. Isolated from soil
N91	<i>Nocardia</i> sp.; Professor H. Veldkamp, A86. Isolated from soil
N136	' <i>Nocardia</i> sp.'; Dr J. Antheunisse, AC86. Isolated from peaty soil
N170, 176	'arthrobacter'; Dr J. Went, ITBON, 11, Kemperbergerweg, Arnhem, Holland, 180, 1845. Isolated from acid forest soil
N180, 184, 185	'arthrobacter'; Dr J. Went, 115, 88b ₂ , 338. Isolated from alkaline forest soil
N242, 245	<i>Nocardia</i> sp.; Dr I. S. Bowie, Department of Microbiology, University of Otago, New Zealand, B32, B48. Isolated from soil
N253 to 256	<i>Nocardia</i> sp.; Dr T. Cross, 193-6. Isolated from Blelham Tarn
N262	<i>Nocardia</i> sp.; Dr T. Cross, CUB364, J. Lacey, A641. Isolated from hay
N273	<i>Nocardia</i> sp.; Dr P. Latter, 30/7, 12.11. Isolated from peat
N277	<i>Mycobacterium</i> sp.; CBS, Myc. 50. Human source
N278	<i>Nocardia</i> sp.; CBS, Carleton. Isolated from Anthoceros sp.
N322	' <i>N. mesenterica</i> ' (Orla-Jensen) Waksman & Henrici; CBS
N348	<i>Nocardia</i> sp.; Dr T. Watson, Hartley Botanical Laboratories, University of Liverpool, England. Isolated from seawater
N350, 351	<i>Nocardia</i> sp.; Dr T. Watson. Isolated from sand dune soil

Table 10 (*cont.*)

Subgroup 14H

N86	<i>Promicromonospora citrea</i> Krassilnikov <i>et al.</i> ; Dr S. T. Williams, E43; N. A. Krassilnikov
N89, 90	' <i>Nocardia</i> sp.'; Isolated from soil
N138, 139	' <i>Nocardia</i> sp.'; Dr J. Antheunisse, R481, R494. Isolated from phyllosphere of grass
N156	<i>Proactinomyces citreus</i> ; Mrs M. Turner, 44
N288	<i>M. xenopei</i> Schwabacher; Dr E. H. Runyon, Veteran's Administration Hospital, Salt Lake City, U.S.A., 2994. Type strain
N297	<i>A. tumescens</i> (Jensen) Conn and Dimmick; NCIB8914; ATCC6947. Cotype (Sneath & Skerman, 1966)

Table 11. *Strains assigned to cluster 15*

N241	<i>Nocardia</i> sp.; Dr I. S. Bowie, B14. Isolated from soil
N243, 244, 246	<i>Nocardia</i> sp.; Dr I. S. Bowie, B40-1, B31. Isolated from soil
N271	<i>Nocardia</i> sp.; Dr P. Latter 4/2 33/14. Isolated from <i>Juncus</i> sp. litter
N294	<i>Mycobacterium fortuitum</i> Cruz; ATCC6841; Cruz. Cotype (Sneath & Skerman, 1966)
N295	<i>Arthrobacter simplex</i> (Jensen) Lochhead; NCIB8929; ATCC6946. Cotype (Sneath & Skerman, 1966)
N352	<i>Nocardia</i> sp.; Dr T. Watson, B19. Isolated from sea water

Table 12. *Strains assigned to the minor clusters*

Cluster 4

N29	<i>Nocardia gardneri</i> (Waksman) Waksman & Henrici; IFO3385; ATCC9604
N68	<i>N. gardneri</i> ; IAM0105

Cluster 6

N203	<i>N. formica</i> Harris & Woodruff; ATCC14811; NRRL2470
N215	<i>N. formica</i> ; Dr J. E. Thiemann, S542; PSA155; M. A. Merck, 143

Cluster 9

N309 to 310	<i>Streptomyces somaliensis</i> (Brumpt) Waksman & Henrici; Professor F. Mariat, 383, 386
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Cluster 11

N33	<i>N. vaccinii</i> Demaree & Smith; National Collection of Plant Pathogenic Bacteria, Harpenden, England, NCPB954; ATCC11092. Holotype (Sneath & Skerman, 1966)
N141	<i>N. vaccinii</i> ; Dr J. Antheunisse; ATCC11092; N. R. Smith, BG19. Holotype (Sneath & Skerman, 1966). Stem galls on <i>Vaccinium</i>

Cluster 12

N1	<i>N. coeliaca</i> (Gray & Thornton) Waksman & Henrici; NCIB9574; R. W. Traxler
N39	<i>N. coeliaca</i> ; NCIB8939; M. Lumb; ATCC13181. Holotype (Sneath & Skerman, 1966)

Cluster 13

N238	<i>N. dassonvillei</i> (Brocq-Rousseau) Liégard and Landrieu; NCTC10489; R. E. Gordon, 1250; D. Taplin; ATCC23219. Isolated from a case of erosive plantar disease
N287	<i>N. dassonvillei</i> ; NCTC10488; R. E. Gordon, 509; P. Thibault
N332	<i>N. dassonvillei</i> ; Professor H. A. Lechevalier, C-CF

Heterogeneous subgroups. The four remaining subgroups were relatively heterogeneous. The named strains in subgroup 14E are *Corynebacterium fascians*, *Nocardia aurantia*, *N. opaca*, *N. restrictus* and a single strain of *Mycobacterium rhodochrous*. Additional strains, isolated from soil, were received as *Nocardia* spp. Subgroup 14F contains soil isolates received as corynebacteria or arthrobacters. Subgroup 14G, defined just above the 80%

S-level, is a large diffuse group. It also contains soil isolates and named strains received as *N. cuniculi*, *N. flava*, *N. mesenterica* and *N. salivae*. The final subgroup, 14H, comprises eight strains loosely associated together. They include two strains of *Promicromonospora citreus*, and the marker strains *Arthrobacter tumescens* and *Mycobacterium xenopei*. The heterogeneous subgroups, as they stand, are of little taxonomic value.

Cluster 15. This cluster is very diffuse and contains a diverse assemblage of organisms (Table 11). Included are the type strains of *Arthrobacter simplex* and *Mycobacterium fortuitum*, and organisms labelled *Nocardia* spp.

Clusters 4, 6, 9, 11, 12 and 13. The minor clusters. Thirteen strains fall into one or other of the six minor clusters (Table 12). Little can be said about clusters which contain so few strains. They are, however, clearly separated from one another and from the established major clusters. The strains found in these clusters may therefore be foci around which future distinct clusters may form. Additional strains of *Nocardia coeliaca*, *N. dassonvillei*, *N. gardneri*, *N. vaccinii* and *Streptomyces somaliensis* will be studied when available.

The ungrouped strains. The 44 strains listed in Table 13 cannot be placed into clusters defined at the 80% S-level. They include the type strains of *Nocardia hydrocarboxydans*, *N. italica*, *N. lurida*, *N. petroleophila*, *N. piedadensis* and *N. saturnea*, as well as strains named *N. alba*, *N. apis*, *N. asteroides*, *N. flavescens*, *N. rugosa* and *N. tenuis*. Some of these strains were undoubtedly misidentified. Most of the marker strains of the genera *Arthrobacter*, *Cellulomonas* and *Mycobacterium* are also unplaced.

Comparison of data using different similarity coefficients and cluster-analysis techniques

The similarity coefficients considered so far included negative matches, and clustering was based on the unweighted average linkage cluster-analysis technique. Many of the strains, particularly *Nocardia asteroides*, *N. madurae* and *N. pelletieri*, gave negative responses to many of the tests. There is a danger that when similarity coefficients are based upon the matching coefficient, strains may be clustered together on negative correlations. The data were therefore re-examined and only positive matches scored. The data obtained from each of the two coefficients of association were analysed by single and unweighted average linkage cluster analysis. The matrices and dendrograms produced are large and too complicated to present here. They are available for consultation from the author.

It was found that both of the cluster-analysis techniques gave very similar results. When only positive matches were counted, however, 18 clusters were formed. They are delineated at the 75% S-level. The discrepancies between the results can be explained.

In the analysis using only positive matches, cluster 3 is not recovered. The two strains of *Nocardia rhodnii*, and the strain of *N. convoluta* and *N. pasteuroides*, respectively form two distinct minor clusters. The other strains which were included in cluster 3 are now unplaced. Subgroup 8A, which contained *N. pelletieri* strains, and subgroup 8B, composed of *Dermatophilus* and *Geodermatophilus*, are also recovered as distinct clusters in the second analysis. In the same way the subgroups of cluster 10 are recovered as two separate clusters. Finally several of the strains originally allocated to cluster 15 are unplaced. It would appear that, as anticipated, some of the high overall similarities found within clusters in the first analysis are based upon negative correlations. The original clusters 3 and 15 are not taxonomically valuable and will not be considered further. In contrast subgroup 8A, containing *N. pelletieri*, and subgroup 10A, composed of *N. turbata* and *N. cellulans*, will be treated as major clusters. The two smaller subgroups, containing *N. marina* and *Dermatophilus congolensis* strains respectively, will be considered as minor clusters.

Most of the remaining subgroups defined by the matching coefficient were recovered

Table 13. *Strains which could not be assigned to any of the clusters*

N291	<i>Arthrobacter globiformis</i> (Conn) Conn & Dimmick; NCIB8602; ATCC4336. Cotype (Sneath & Skerman, 1966)
N296	<i>A. ureafaciens</i> (Krebs & Eggleston) Clark; NCIB7811; NCTC7811; ATCC7562. Holo-type (Sneath & Skerman, 1966)
N177, 178	* <i>Arthrobacter</i> sp.; Dr J. Went. From alkaline forest soil
N293	<i>Cellulomonas biazotea</i> (Kellerman <i>et al.</i>) Bergey <i>et al.</i> , NCIB8077; ATCC486; N. R. Smith, 127. Cotype (Sneath & Skerman, 1966)
N290	<i>Mycobacterium phlei</i> Lehmann & Neumann; NCTC8151; G. Penso; ATCC19249. Suggested working type (Sneath & Skerman, 1966)
N292	<i>M. smegmatis</i> (Trevisan) Lehmann & Neumann; NCTC8159; R. E. Gordon; W. Hagan; Alvarez and Tavel. Cotype (Sneath & Skerman, 1966)
N6	<i>Nocardia alba</i> (Rossi-Doria) Chalmers & Christopherson; NCIB9702; K. Wuhrmann
N235	<i>N. alba</i> Schoen; Dr I. Uesaka, Kyoto University, Kyoto, Japan, 113; N. M. McClung, 113; R. E. Gordon, 506
N8	<i>N. apis</i> ; NCIB9378; A. di Marco. Isolated from the intestine of <i>Apis mellifera</i>
N264	<i>N. sp. asteroides</i> type; Dr T. Cross, CUB412; J. Lacey, A642. Isolated from straw
N345	<i>N. asteroides</i> ; Public Health Laboratories, Leicester, England
N347	<i>N. flavescens</i> (Jensen) Waksman & Henrici; CBS, strain Hoffman-La Roche
N42	<i>N. hydrocarboxydans</i> Nolof & Hirsch; NCIB9436, strain Hirsch 70. ATCC15104
N9	<i>N. italica</i> Spalla, Amici, Bianchi; NCIB9386
N323	<i>N. keratolytica</i> (Acton & McGuire) Reiss & Briggs; ATCC12484
N2	<i>N. lurida</i> ; NCIB9601
N69	<i>N. narashinoensis</i> Endo; IAM0113
N315	<i>N. paraguayensis</i> Almeida; Professor F. Mariat, 355; IHM951 (<i>Actinomyces paraguayensis</i>)
N43	<i>N. petroleophila</i> Hirsch & Engel; NCIB9438, strain Hirsch; ATCC15777. Type strain. From soil
N207	<i>N. piedadensis</i> Castellani; ATCC15747; A. Castellani. Type strain. Probable cause of <i>Macrouleus perstans</i>
N320	<i>N. piedadensis</i> ; Professor A. Castellani, Institute of Tropical Medicine, Lisbon, Portugal
N237	<i>N. pretoriana</i> Pijper & Pullinger; Dr I. Uesaka, 194; N. M. McClung; J. D. Schneidau, Jun.; 368. Isolated from mycetoma of chest
N44	<i>N. rugosa</i> A. di Marco; NCIB8926
N45	<i>N. saturnea</i> Hirsch; NCIB9437, strain Hirsch 71; ATCC15809. Type strain. Isolated as an air contaminant
N319	<i>N. saturnea</i> ; ATCC15778; P. Hirsch, 99. Type strain. Isolated from compost soil
N117	<i>N. tenuis</i> Castellani; CBS260.35
N236	<i>N. transvalensis</i> Pijper and Pullinger; Dr I. Uesaka, 193; N. M. McClung, 193; J. D. Schneidau, Jun.; 2392; R. E. Gordon, 2392. Isolated from mycetoma of foot
N82	<i>Nocardia</i> sp.; Dr S. T. Williams, E32
N202	<i>Nocardia</i> sp.; Leicester PHLS. Isolated from sputum
N258	<i>Nocardia</i> sp.; Dr T. Cross, CUB353; J. Lacey, A321. Isolated from moist barley grain
N247	<i>Nocardia</i> sp.; Dr I. S. Bowie, B42. From soil
N259	<i>Nocardia</i> sp.; Dr T. Cross, CUB355; J. Lacey, A333. Isolated from hay
N260, 261	<i>Nocardia</i> sp.; Dr T. Cross, CUB359, 360; J. Lacey, A409, A410. Isolated from moist barley grain
N349, 353, 355	<i>Nocardia</i> sp.; Dr T. Watson, A27, B24, B100. Isolated from beach sandy soil
N132	<i>Proactinomyces actinoides</i> (Smith) Krassilnikov; Professor V. D. Kuznetsov, U.S.S.R. Research Institute for Antibiotics, Moscow, RIA644
N133	<i>P. gabritschewski</i> (Berestnev) Krassilnikov; Professor V. D. Kuznetsov, RIA642.
N134	<i>P. tenuis</i> (Castellani) Krassilnikov; Professor V. D. Kuznetsov; RIA638
N88	<i>Streptomyces bobilliae</i> (Waksman and Curtis) Waksman & Henrici; Dr S. T. Williams, A34
N20	<i>S. somaliensis</i> ; NCTC3236

when only positive matches were scored. They include the subgroups of cluster 2, as well as four of the five subgroups of the *Nocardia asteroides* cluster. The single exception, subgroup 1 D, is placed at the periphery of subgroup 1 A in the second analysis. With cluster 14 subgroups 14 E and 14 F, known to be diffuse, are not recovered in their entirety. Most of these subgroups are, therefore, taxonomically significant. This is not so with the subgroups of cluster 7. In the second analysis well-defined subgroups of *N. madurae* strains are not found.

Description of clusters obtained by using only positive matches.

In the extensive phenotypic characterization of strains several characters were found to have a descriptive or discriminatory value. This was readily apparent when the frequency of each test which was positive in each of the clusters had been calculated (Table 14). Some features were common to almost all of the clustered strains. They were all aerobic, Gram-positive, formed a primary mycelium, grew on YEA at 25° to 40°, at reactions ranging from pH 6 to pH 8, and they were not inhibited by 0.000001 % (w/v) crystal violet, 0.001 % (w/v) sodium azide, 3 % (w/v) sodium chloride, 0.001 % (w/v) potassium tellurite, 0.001 % (v/v) Teepol or 0.1 % (w/v) tetrazolium. Strains were benzidine- and catalase-positive, reduced nitrate, produced acid from glucose but not from dulcitol, inulin, melezitose or raffinose, and used sodium acetate, butyrate, propionate and paraffin as a sole source of carbon and energy.

Cluster 1. These strains formed a mycelium which fragmented into rods and cocci, produced a stroma which was usually pink or orange and carried at least a trace of white aerial hyphae. Most of the strains showed some degree of acid-fastness, nearly half produced a melanin-like pigment, and nearly all had irregular colonies with filamentous margins. They degraded urea and allantoin, were resistant to lysozyme, to 5 % (w/v) sodium chloride, and to the lower concentrations of the other inhibitors used. They hydrolysed aesculin and Tweens 20, 40 and 60, and produced acid from D-fructose, mannose, arbutin, dextrin and glycerol though generally their fermentative activity was limited. The screening of carbon and energy sources revealed unrecognized nutritional characters. For instance, most strains were able to grow well on sebatic acid and testosterone, and more than half on adipic acid, as well as on sodium-*H*-malate, pyruvate and succinate. Alternatively, the strains were unable to use amino or aromatic acids, phenol or phenolic derivatives as sole carbon sources.

Cluster 2. All strains in this cluster formed a mycelium which fragmented, were acid-fast, produced a brown exopigment and formed aerial hyphae which only rarely covered the creamy/white stroma. The colonies were irregular and margins filamentous. Aesculin, guanine, hypoxanthine, xanthine and most of the Tweens were hydrolysed and acid formed from D-fructose, mannose, arbutin, glycerol, mannitol and inositol. The strains grew in the presence of 7 % (w/v) sodium chloride, 0.01 % (w/v) bismuth citrate and 0.05 % (w/v) potassium tellurite. The spectrum of activity towards the biochemical and nutritional tests mirrored that shown by *Nocardia asteroides* strains.

Cluster 5. *Nocardia brasiliensis* strains formed a mycelium which fragmented into rods and cocci, produced a brown exopigment, were strongly acid-fast and produced aerial hyphae which frequently covered the orange/brown stroma in a white down. They were fermentatively active and acid was produced from D-arabinose, D-galactose, D-fructose, mannose, trehalose, arbutin, glycerol, mannitol and inositol. Exocellular proteases degraded casein, gelatin and keratin; aesculin, deoxyribonucleic acid, guanine, hypoxanthine and tyrosine were also readily degraded. Strains in this cluster had similar biochemical and nutritional properties to *N. asteroides* and *N. caviae*, but unlike them utilized sodium gluconate, L-proline and L-tyrosine as sole sources of carbon and energy.

Cluster 7. The strains in this cluster were not acid-fast, grew slowly producing a mycelium which generally did not fragment, nearly half formed a brown exopigment, but only a few formed aerial hyphae, and even then only feebly. The colonies were white/cream and the margins deeply filamentous. The *Nocardia madurae* strains formed acid from D-xylose, L-rhamnose, D-galactose, maltose, trehalose, arbutin, mannitol and less readily from adonitol and mannose. Casein, gelatin, keratin, aesculin, hypoxanthine, tyrosine and Tweens 20, 40 and 60 were readily degraded; adenine, guanine and deoxyribonucleic acid less so. Urea was also broken down but the strains showed no distinctive biochemical or nutritional properties.

Cluster 8 A. The *Nocardia pelletieri* strains were slow growers, difficult to cultivate, formed a permanent mycelium and occasionally a characteristic red exopigment. Aerial hyphae were only feebly produced, and under strongly aerobic conditions a reddish, frequently deep red, stroma, though white mutants were not uncommon. Acid was produced from trehalose but from few other sugars, and casein, gelatin, keratin, deoxyribonucleic acid, hypoxanthine, tyrosine and chitin were degraded. The organisms grew on a range of carbon sources which included sebatic acid, sodium-*H*-malate, pyruvate, gluconate, citrate and L-proline but were inhibited by lysozyme and low concentrations of bismuth citrate, thallos acetate, tetrazolium and penicillin.

Cluster 10 A. The *Nocardia turbata* strains were non-acid fast and formed a primary mycelium which fragmented into flagellated motile rods. The colonies were smooth, yellow, convex and had deeply filamentous margins. They were very active fermentatively, produced acid from glucose anaerobically and from most of the sugars and sugar alcohols examined, including cellobiose, sucrose, glycogen and amygdalin. Aesculin, casein, gelatin, keratin, chitin, deoxyribonucleic acid and Tweens 20, 40 and 60 were degraded and urea and allantoin broken down. The carbon utilization pattern was limited though growth occurred on sodium lactate and gluconate, and readily in media containing 7% (w/v) sodium chloride, 0.4% (v/v) phenyl ethanol, 0.1% (w/v) phenol and 0.01% (w/v) bismuth citrate.

Cluster 14. Most of the strains in this large cluster formed a primary mycelium which soon fragmented into rods and cocci; aerial hyphae when produced were short and sparse. A few strains were weakly acid-fast, and most colonies were pink, orange or red, though this property was not stable. Acid was readily produced from maltose, D-fructose, mannose and glycerol and less so from sucrose, trehalose, arbutin and sorbitol. Most strains degraded Tweens 20, 40 and 60 and approximately half adenine, deoxyribonucleic acid, tyrosine, urea and allantoin. Sodium-D-tartrate and pyruvate were utilized by most of the organisms though a wide range of carbon sources were used by the cluster as a whole. The *Mycobacterium rhodochrous* strains were sensitive to lysozyme and penicillin but grew in the presence of 5% (w/v) sodium chloride, 0.01% (w/v) sodium azide, 0.4% (v/v) phenyl ethanol and 0.01% (w/v) potassium tellurite.

The minor clusters. Unfortunately these clusters were represented by only two or three strains so that the weight which can be attached to character attributes they show is uncertain. Nevertheless the characters which might be important in describing these groups will be mentioned. The *Nocardia gardneri* strains of cluster 4 shared a relatively high general similarity with *N. asteroides* and this was reflected in their properties. They were acid-fast, formed a mycelium which fragmented, produced a brown exopigment and a moderate amount of white aerial hyphae. They formed acid from D-fructose, mannose, sucrose, maltose, arbutin, glycerol, sorbitol and dextrin and degraded Tweens 20, 40 and 60, and urea and allantoin. They grew in media which contained 3% (w/v) sodium chloride, 0.1% (w/v) phenol and the lower concentration of the other inhibitors, and utilized sodium acetate, citrate, butyrate, succinate, adipic and sebatic acids as sole carbon sources.

The *Nocardia formica* strains of cluster 6 formed a mycelium which fragmented, a brown exopigment, a moderate amount of aerial hyphae; they were not acid-fast but were active fermentatively and biochemically. Nitrate, allantoin and urea were decomposed, and acid formed from D-fructose, D-galactose, lactose, maltose, trehalose, glycogen and inositol. Adenine, casein, gelatin, keratin, deoxyribonucleic acid, guanine, hypoxanthine and Tweens 20, 40 and 60 were degraded, and the compounds listed for cluster 4 utilized as sole carbon sources.

The two *Streptomyces somaliensis* strains in cluster 9 had a permanent mycelium, were non-acid-fast and produced abundant white aerial hyphae some of which carried long chains of spores. Acid was produced from mannose, maltose and dextrin, and Tweens 20, 40 and 60, casein, gelatin, keratin and deoxyribonucleic acid were degraded. The organisms were sensitive to bismuth citrate, penicillin and lysozyme, and utilized sodium acetate, citrate, propionate, L-tyrosine and paraffin as sole sources of carbon.

Cluster 11 contains the *Nocardia vaccinii* strains, which were acid-fast, formed a mycelium which fragmented, produced an exopigment, and a red stroma covered with white aerial hyphae. Acid was produced from L-arabinose, D-xylose, L-rhamnose, D-fructose, D-galactose, mannose, cellobiose, sucrose, trehalose, glycerol and mannitol. Cellulose, chitin, deoxyribonucleic acid, urea and Tweens 20, 40 and 60 were degraded. These strains were sensitive to penicillin, resistant to lysozyme and used sodium lactate, *H*-malate, malonate and octoate, as well as more commonly used carbon compounds.

The *Nocardia coeliaca* strains of cluster 12 were acid-fast, produced a mycelium which fragmented, abundant aerial hyphae and a brown diffusible exopigment. They formed acid from maltose, melezitose, salicin and ethanol as well as from all the sugars attacked by *N. vaccinii*. Adenine, gelatin, casein, guanine, hypoxanthine, tyrosine and xanthine were degraded. The strains broke down urea and allantoin, were not inhibited by lysozyme or penicillin, and utilized as sole carbon sources all the compounds used by *N. asteroides* and *N. vaccinii*.

The three *Nocardia dassonvillei* strains in cluster 13 were not acid-fast, formed a mycelium which fragmented into rods and cocci, produced an exopigment and abundant aerial hyphae some of which segmented into chains of spores. Acid was formed from L-arabinose, D-xylose, L-rhamnose, D-fructose, D-galactose, mannose, cellobiose, sucrose, maltose, glycogen, arbutin, glycerol and mannitol. All the organic compounds were hydrolysed with the exception of cellulose, chitin, Tween 80 and xylan. The organisms were sensitive to lysozyme and penicillin, and utilized a wide range of carbon sources as sole source of carbon, including sodium lactate and tartrate.

The four remaining minor clusters are those which were defined in the second computer analysis when positive matches alone were considered. One of them contains the *Dermatophilus* strains, which were unreactive to most of the tests but were recognized by the way in which the hyphae liberated motile coccoid elements. They degraded casein, gelatin, keratin, urea and allantoin.

The *Nocardia marina* strains were nonacid-fast, formed a mycelium which fragmented and produced yellow convex colonies with entire margins. Acid was produced from D-fructose, mannose, sucrose, maltose, trehalose, glycogen, mannitol and sorbitol, and adenine, cellulose, chitin, gelatin, keratin and Tweens 20, 40 and 60 degraded. They reduced nitrate, produced urease and allantoinase, grew on sodium acetate, propionate and lactate, and in the presence of 10% (w/v) sodium chloride but were sensitive to penicillin and lysozyme.

The *Nocardia rhodnii* strains had many characters in common with *N. asteroides*. They were nonacid-fast, had a mycelium which fragmented and formed an orange stroma which

Table 14. *Percentage frequency of positive characters found in major clusters defined at the 80% similarity level*

		<i>Nocardia</i>	<i>N.</i>	<i>N. bra-</i>	<i>N.</i>	<i>N. pel-</i>	<i>N.</i>	<i>Mycobac-</i>
		<i>asteroides</i>	<i>caviae</i>	<i>siliensis</i>	<i>madurae</i>	<i>letieri</i>	<i>turbata</i>	<i>terium</i>
								<i>rhodoch-</i>
								<i>rous</i>
Cluster	...	1	2	5	7	8A	10A	14
No. of strains tested	...	42	10	10	22	11	5	104
1	Mycelium, formed	100	100	100	100	100	100	81
2	Mycelium, temporary	100	100	100	27	0	100	100
3	Rods and cocci found	100	100	100	27	0	100	96
4	Cocci only	0	0	0	0	0	0	4
5	Rods only	0	0	0	0	0	0	0
6	Acid-fastness, weak	70	100	100	0	0	0	19
7	Acid-fastness, strong	46	50	100	0	0	0	10
8	Strains motile	0	0	0	0	0	80	0
9	Aerial hyphae, sparse	98	90	100	23	72	0	39
10	Aerial hyphae, moderate	77	60	100	14	9	0	7
11	Aerial hyphae, abundant	62	20	100	9	9	0	2
12	Colonies, white/grey	17	100	0	100	9	0	36
13	Colonies, pink/orange/red	77	0	70	0	90	0	57
14	Colonies, black	0	0	0	0	0	0	0
15	Colonies, brown	7	0	30	0	0	0	0
16	Colonies, yellow	0	0	0	0	0	100	6
17	Exopigment, water soluble	43	90	100	41	36	0	6
18	Colony margin, entire	0	0	0	0	0	0	73
19	Colony margin, filamentous	48	100	100	2	100	20	20
20	Colony margin, deeply filamentous	52	0	0	98	0	80	7
21	Colonies easy to emulsify	0	30	20	0	0	100	89
22	Suspension, coarse	100	100	100	100	100	0	16
23	Colony elevation, flat/raised	0	0	0	0	0	0	12
24	Colony elevation, convex	12	20	0	0	0	100	65
25	Colony elevation, irregular	89	80	100	100	100	0	23
Fermentation studies								
Acid from:								
26	L-Arabinose	0	0	0	45	0	100	11
27	D-Arabinose	0	0	100	0	0	0	0
28	D-Xylose	0	0	0	100	0	80	5
29	L-Rhamnose	24	0	0	85	0	0	2
30	D-Fructose	98	100	100	21	54	100	89
31	D-Galactose	17	0	100	85	0	80	15
32	Glucose, oxidative	98	100	100	100	90	100	92
33	Glucose, fermentative	0	0	0	0	0	100	0
34	Mannose	86	40	100	50	0	100	84
35	Cellobiose	5	0	0	0	0	100	11
36	Lactose	0	0	0	0	0	80	5
37	Sucrose	0	0	0	0	0	100	71
38	Maltose	53	50	0	91	18	100	84
39	Trehalose	55	50	100	77	81	100	67
40	Melezitose	0	0	0	0	0	0	4
41	Raffinose	0	0	0	0	0	20	6
42	Glycogen	0	0	0	0	0	100	5
43	Inulin	0	0	0	0	0	0	0
44	Amygdalin	0	0	0	0	0	100	9
45	Arbutin	89	100	90	86	0	100	48
46	Salicin	17	0	10	0	0	100	22
47	Adonitol	41	0	0	59	0	0	3
48	Dulcitol	0	0	0	0	0	0	0
49	Glycerol	95	100	100	85	0	100	82

Table 14 (cont.)

			<i>Nocardia</i>	<i>N.</i>	<i>N. bra-</i>	<i>N.</i>	<i>N. pel-</i>	<i>Mycobac-</i>
			<i>asteroides</i>	<i>caviae</i>	<i>siliensis</i>	<i>madurae</i>	<i>letieri</i>	<i>terium</i>
								<i>rhodoch-</i>
								<i>turbata</i>
								<i>rous</i>
Cluster	...		1	2	5	7	8A	10A
No. of strains tested	...		42	10	10	22	11	5
50	Mannitol		7	80	90	85	9	0
51	Sorbitol		2	10	0	0	0	100
52	<i>meso</i> -Inositol		0	100	100	18	0	0
53	Ethanol		24	0	0	0	0	0
54	Dextrin		93	40	0	85	72	100
Hydrolysis tests								
55	Adenine, strong		0	0	0	23	0	0
56	Adenine, weak		0	0	0	23	0	0
57	Aesculin		93	100	100	100	0	100
58	Casein, strong		0	0	60	59	90	40
59	Casein, weak		0	0	100	81	100	100
60	Cellulose		0	0	0	5	0	20
61	Chitin		0	0	0	5	90	100
62	DNA		0	30	100	72	90	100
63	Gelatin, strong		0	0	80	59	100	100
64	Gelatin, weak		0	0	100	100	100	100
65	Guanine		0	90	90	63	0	0
66	Hippurate		0	0	0	0	0	0
67	Hypoxanthine, strong		5	90	90	45	54	20
68	Hypoxanthine, weak		5	90	100	81	100	20
69	Keratin, strong		0	0	60	18	81	80
70	Keratin, weak		0	0	100	81	100	80
71	Tween 20		100	90	20	100	20	100
72	Tween 40		97	70	10	100	9	100
73	Tween 60		84	70	10	100	9	100
74	Tween 80		10	20	0	14	0	0
75	Tyrosine, strong		0	0	70	72	54	0
76	Tyrosine, weak		0	0	100	91	100	0
77	Xanthine, strong		0	40	0	0	0	20
78	Xanthine, weak		0	100	0	0	0	20
79	Xylan		0	0	0	0	0	20
Sole carbon source								
80	L-Arabinose		0	0	50	59	72	100
81	D-Arabinose		0	0	20	0	0	0
82	D-Xylose		0	0	30	91	0	100
83	L-Rhamnose		36	0	0	77	9	0
84	D-Fructose		91	100	80	63	0	100
85	D-Galactose		24	0	90	68	63	80
86	D-Glucose		100	100	100	100	100	100
87	Mannose		86	40	100	50	9	100
88	Cellobiose		0	30	0	18	0	100
89	Lactose		0	0	10	0	0	100
90	Sucrose		7	20	70	14	0	100
91	Maltose		74	60	100	81	18	100
92	Trehalose		26	40	100	95	90	100
93	Melezitose		0	0	0	0	0	80
94	Raffinose		0	0	0	0	0	20
95	Glycogen		0	0	10	9	63	100
96	Inulin		0	0	0	0	0	0
97	Amygdalin		5	0	0	0	0	100
98	Arbutin		0	60	50	14	0	0

Table 14 (*cont.*)

			<i>Nocardia</i>	<i>N.</i>	<i>N. bra-</i>	<i>N.</i>	<i>N. pel-</i>	<i>N.</i>	<i>Mycobac-</i>
			<i>asteroides</i>	<i>caviae</i>	<i>siliensis</i>	<i>madurae</i>	<i>letieri</i>	<i>turbata</i>	<i>terium</i>
									<i>rhodoch-</i>
									<i>rous</i>
Cluster	...		1	2	5	7	8A	10A	14
No. of strains tested	...		42	10	10	22	11	5	104
99	Salicin		10	20	30	0	0	100	33
100	Adonitol		0	0	0	68	0	0	3
101	Dulcitol		0	0	0	0	0	0	7
102	Glycerol		82	10	90	81	45	100	80
103	Mannitol		53	100	90	81	9	20	73
104	Sorbitol		7	10	0	0	0	100	62
105	<i>meso</i> -Inositol		2	90	100	5	0	0	21
106	Dextrin		50	50	10	40	0	100	35
107	Starch		41	40	50	14	9	100	47
108	Glucosamine		0	50	90	28	0	40	37
109	Sodium acetate		100	100	100	81	100	100	79
110	Sodium propionate		100	80	100	68	100	100	89
111	Sodium- <i>n</i> -butyrate		100	100	100	72	100	0	83
112	Sodium- <i>n</i> -octoate		0	0	0	0	0	0	35
113	Sodium malonate		0	0	0	0	0	0	0
114	Sodium benzoate		12	0	0	0	0	0	27
115	Sodium succinate		100	100	80	81	63	0	73
116	Adipic acid		53	0	0	0	0	0	56
117	Pimelic acid		0	0	10	0	9	0	42
118	Sebacic acid		84	100	90	0	90	0	54
119	Sodium lactate		0	0	0	0	0	100	55
120	Sodium- <i>H</i> -malate		98	100	100	68	100	0	73
121	Sodium-D-tartrate		0	0	0	0	0	0	90
122	Sodium citrate		12	50	90	45	90	0	69
123	Sodium hippurate		0	0	0	0	0	0	2
124	D- α -Alanine		0	0	10	9	9	0	41
125	L- α -Alanine		0	60	50	9	36	0	40
126	DL-nor-Leucine		0	0	0	0	0	0	36
127	L-Threonine		0	0	0	0	0	0	9
128	L-Serine		0	50	40	0	27	0	27
129	L-Tryptophan		0	0	0	0	0	0	0
130	L-Proline		0	0	90	18	90	0	31
131	L-Tyrosine		0	0	70	9	54	0	31
132	<i>p</i> -Hydroxybenzoic acid		0	0	0	0	0	0	43
133	<i>m</i> -Hydroxybenzoic acid		14	0	0	0	0	0	31
134	D-Mandelic acid		0	0	0	0	0	0	1
135	Protocatechuic acid		7	0	30	0	27	20	60
136	Testosterone		82	100	0	0	0	0	57
137	Sodium pyruvate		100	100	100	50	100	100	83
138	Betaine HCl		0	0	0	0	0	0	2
139	Acetamide		7	10	0	0	0	0	58
140	Benzamide		0	0	0	0	0	0	8
141	<i>p</i> -Cresol		2	10	0	0	0	0	33
142	Paraffin		100	90	100	100	90	100	84
143	Sodium gluconate		0	0	90	55	81	100	62
144	<i>o</i> -Hydroxybenzaldehyde		0	0	0	0	0	0	23
Sole carbon and nitrogen source									
145	Trimethyldiamine		0	0	0	5	0	0	2
146	Serine		0	0	70	0	0	0	0
147	Acetamide		14	0	50	14	0	0	31

Table 14 (cont.)

		<i>Nocardia</i> <i>asteroides</i>	<i>N.</i> <i>caviae</i>	<i>N. bra-</i> <i>siliensis</i>	<i>N.</i> <i>madurae</i>	<i>N. pel-</i> <i>letieri</i>	<i>N. tur-</i> <i>bata</i>	<i>Mycobac-</i> <i>terium</i> <i>rhodoch-</i> <i>rous</i>
Cluster	...	1	2	5	7	8A	10A	14
No. of strains tested		42	10	10	22	11	5	104
Inhibition tests								
Growth on YEA containing:								
148	3 % (w/v) NaCl	100	100	100	100	100	100	94
149	5 % (w/v) NaCl	84	100	100	72	100	100	83
150	7 % (w/v) NaCl	36	80	0	40	0	100	69
151	10 % (w/v) NaCl	0	0	0	9	0	0	9
152	0.001 % (w/v) sodium azide	100	100	100	100	100	100	100
153	0.01 % (w/v) sodium azide	62	40	30	50	9	100	83
154	0.02 % (w/v) sodium azide	12	0	0	0	0	80	54
155	0.03 % (w/v) sodium azide	0	0	0	0	0	60	2
156	0.001 % (w/v) potassium tellurite	100	100	100	100	100	100	95
157	0.01 % (w/v) potassium tellurite	79	80	30	59	27	60	88
158	0.05 % (w/v) potassium tellurite	60	70	20	23	18	0	62
159	0.1 % (w/v) potassium tellurite	41	10	0	9	0	0	38
160	0.1 % (v/v) phenyl ethanol	89	100	70	100	54	100	97
161	0.2 % (v/v) phenyl ethanol	43	60	50	59	36	100	90
162	0.3 % (v/v) phenyl ethanol	22	40	20	27	18	100	79
163	0.4 % (v/v) phenyl ethanol	0	10	0	0	0	100	71
164	0.000001 % (w/v) crystal violet	100	100	100	91	100	100	91
165	0.00001 % (w/v) crystal violet	84	100	100	63	100	100	89
166	0.0001 % (w/v) crystal violet	38	50	10	0	9	0	48
167	0.001 % (w/v) crystal violet	2	10	0	0	0	0	11
168	0.001 % (v/v) Teepol	95	100	100	100	100	100	97
169	0.005 % (v/v) Teepol	75	100	100	95	100	100	90
170	0.01 % (v/v) Teepol	70	60	80	36	0	100	77
171	0.001 % (w/v) phenol	100	100	100	100	27	100	98
172	0.01 % (w/v) phenol	86	90	100	54	0	100	86
173	0.1 % (w/v) phenol	43	50	30	14	0	100	46
174	0.0001 % (w/v) bismuth citrate	95	100	100	72	0	100	95
175	0.001 % (w/v) bismuth citrate	90	100	100	27	0	100	83
176	0.01 % (w/v) bismuth citrate	43	90	100	5	0	100	39
177	0.0001 % (w/v) thallous acetate	100	100	90	91	18	100	91
178	0.001 % (w/v) thallous acetate	5	20	0	5	0	100	19
179	0.01 % (w/v) thallous acetate	0	0	0	0	0	80	2
180	0.01 % (w/v) tetrazolium	95	80	100	22	0	100	90
181	0.1 % (w/v) tetrazolium	0	0	0	0	0	0	2
182	Sensitivity to penicillin	24	30	10	81	100	100	98
Growth at:								
183	pH 5	2	0	0	0	0	0	2
184	pH 6	98	100	100	72	100	40	94
185	pH 8	100	100	100	81	81	100	95
186	pH 9	98	100	100	50	27	100	86
187	pH 10	62	100	30	23	0	20	79
188	50°	31	10	0	5	27	0	3
189	45°	64	60	20	45	90	0	9
190	40°	100	90	90	100	100	100	90
191	35°	100	100	100	100	100	100	89
192	10°	26	30	60	0	9	100	83

Table 14 (*cont.*)

		<i>Nocardia asteroides</i>	<i>N. caviae</i>	<i>N. bra- siliensis</i>	<i>N. madurae</i>	<i>N. pel- letieri</i>	<i>N. turbata</i>	<i>Mycobac- terium rhodoch- rous</i>
Cluster	...	1	2	5	7	8A	10A	14
No. of strains tested	...	42	10	10	22	11	5	104
Biochemical tests								
193	Benzidine, strong	19	80	60	100	90	20	90
194	Benzidine, weak	100	80	100	100	100	100	93
195	Oxidase	5	0	0	9	0	20	4
196	Catalase, strong	54	80	60	100	81	80	81
197	Nitrate reduction	98	100	100	81	100	100	87
198	Urease, strong	79	100	100	100	14	100	43
199	Urease, weak	98	100	100	100	14	100	50
200	Allantoinase, strong	24	50	70	0	0	0	7
201	Allantoinase, weak	86	100	100	0	0	100	35
202	Lysozyme resistant	100	100	100	0	0	0	3
203	<i>p</i> -Nitrophenoloxidase	14	0	0	0	0	0	6
204	<i>m</i> -Nitrophenoloxidase	0	0	0	0	0	0	10

Table 15. *Characters which serve to differentiate major clusters*

		<i>Nocardia asteroides</i>	<i>N. caviae</i>	<i>N. bra- siliensis</i>	<i>N. mad- urae</i>	<i>N. pel- letieri</i>	<i>N. turbata</i>	<i>Mycobac- terium rhodoch- rous</i>
Cluster	...	1	2	5	7	8A	10A	14
No. of strains tested	...	42	10	10	22	11	5	104
2	Mycelium, temporary	+	+	+	<i>d</i>	—	+	+
6	Acid-fastness, weak	<i>d</i>	+	+	—	—	—	—
8	Strains motile	—	—	—	—	—	+	—
9	Aerial hyphae, sparse	+	+	+	<i>d</i>	<i>d</i>	—	<i>d</i>
16	Colonies, yellow	—	—	—	—	—	+	—
17	Exopigment, water soluble	<i>d</i>	+	+	<i>d</i>	<i>d</i>	—	—
Fermentation studies								
Acid from:								
26	L-Arabinose	—	—	—	<i>d</i>	—	+	—
27	D-Arabinose	—	—	+	—	—	—	—
28	D-Xylose	—	—	—	+	—	+	—
29	L-Rhamnose	<i>d</i>	—	—	+	—	—	—
31	D-Galactose	—	—	+	+	—	+	—
33	D-Glucose, fermentative	—	—	—	—	—	+	—
35	Cellobiose	—	—	—	—	—	+	—
36	Lactose	—	—	—	—	—	+	—
37	Sucrose	—	—	—	—	—	+	<i>d</i>
38	Maltose	<i>d</i>	<i>d</i>	—	+	—	+	+
42	Glycogen	—	—	—	—	—	+	—
44	Amygdalin	—	—	—	—	—	+	—
46	Salicin	—	—	—	—	—	+	<i>d</i>
49	Glycerol	+	+	+	+	—	+	+
50	Mannitol	—	+	+	+	—	—	<i>d</i>
51	Sorbitol	—	—	—	—	—	—	<i>d</i>
52	<i>meso</i> -Inositol	—	+	+	—	—	—	—

Table 15 (cont.)

Cluster ...	<i>Nocardia asteroides</i>	<i>N. caviae</i>	<i>N. bra- siliensis</i>	<i>N. mad- urae</i>	<i>N. pel- letieri</i>	<i>N. turbata</i>	<i>Mycobac- terium rhodoch- rous</i>
No. of strains tested ...	1	2	5	7	8A	10A	14
	42	10	10	22	11	5	104
Hydrolysis tests							
59 Casein, weak	—	—	+	+	+	+	—
61 Chitin	—	—	—	—	+	+	—
62 DNA	—	<i>d</i>	+	<i>d</i>	+	+	<i>d</i>
65 Guanine	—	+	+	<i>d</i>	—	—	—
68 Hypoxanthine, weak	—	+	+	+	+	—	—
70 Keratin, weak	—	—	+	+	+	+	—
72 Tween 40	+	<i>d</i>	—	+	—	+	+
75 Tyrosine, weak	—	—	+	+	+	—	<i>d</i>
78 Xanthine, weak	—	+	—	—	—	—	—
Sole carbon source							
82 D-Xylose	—	—	<i>d</i>	+	—	+	<i>d</i>
85 D-Galactose	<i>d</i>	—	+	<i>d</i>	<i>d</i>	+	<i>d</i>
87 Mannose	+	<i>d</i>	+	<i>d</i>	—	+	+
88 Cellobiose	—	<i>d</i>	—	—	—	+	<i>d</i>
89 Lactose	—	—	—	—	—	+	—
90 Sucrose	—	<i>d</i>	<i>d</i>	—	—	+	<i>d</i>
93 Melezitose	—	—	—	—	—	+	—
95 Glycogen	—	—	—	—	<i>d</i>	+	—
97 Amygdalin	—	—	—	—	—	+	—
99 Salicin	—	<i>d</i>	<i>d</i>	—	—	+	<i>d</i>
103 Mannitol	<i>d</i>	+	+	+	—	<i>d</i>	<i>d</i>
104 Sorbitol	—	—	—	—	—	+	<i>d</i>
105 meso-Inositol	—	+	+	—	—	—	<i>d</i>
108 Glucosamine	—	<i>d</i>	+	<i>d</i>	—	<i>d</i>	<i>d</i>
111 Sodium- <i>n</i> -butyrate	+	+	+	<i>d</i>	+	—	+
115 Sodium succinate	+	—	+	+	<i>d</i>	—	<i>d</i>
118 Sebacic acid	+	—	+	—	+	—	<i>d</i>
119 Sodium lactate	—	—	—	—	—	+	<i>d</i>
120 Sodium- <i>H</i> -malate	+	+	+	<i>d</i>	+	—	<i>d</i>
121 Sodium tartrate	—	—	—	—	—	—	+
122 Sodium citrate	—	<i>d</i>	+	<i>d</i>	+	—	<i>d</i>
130 L-Proline	—	—	+	<i>d</i>	+	—	<i>d</i>
136 Testosterone	+	+	—	—	—	—	<i>d</i>
143 Sodium gluconate	—	—	+	<i>d</i>	+	+	<i>d</i>
Inhibition tests							
150 7% (w/v) sodium chloride	<i>d</i>	+	—	<i>d</i>	—	+	<i>d</i>
163 0.4% (w/v) phenyl ethanol	—	—	—	—	—	+	<i>d</i>
172 0.01% (w/v) phenol	+	+	+	<i>d</i>	—	+	+
177 0.001% (w/v) thallos acetate	+	+	+	+	—	+	+
182 Sensitivity to penicillin	<i>d</i>	<i>d</i>	—	+	+	+	+
Biochemical tests							
199 Urease, weak	+	+	+	+	—	+	<i>d</i>
201 Allantoinase, weak	+	+	+	—	—	+	<i>d</i>
202 Lysozyme resistant	+	+	+	—	—	—	—

showed no trace of aerial hyphae. They produced acid from D-fructose, mannose, sucrose, maltose, trehalose, mannitol, sorbitol and ethanol, reduced nitrate, and degraded allantoin, urea and Tweens 20, 40, 60 and 80. They used sodium acetate, *n*-butyrate, propionate, *n*-octoate, *H*-malate, tartrate, adipic and sebacic acid as sole sources of carbon and energy but were inhibited by penicillin and lysozyme.

The final minor cluster contains *Nocardia pasteuroides* and *N. convoluta* (N95). These strains formed a mycelium which fragmented, and an orange stroma covered with white aerial hyphae. They formed acid from D-fructose, D-galactose, mannose, glycerol and mannitol, reduced nitrate and degraded Tweens 20, 40 and 60. The strains were resistant to lysozyme and penicillin and used sodium acetate, *n*-butyrate, propionate, succinate, pimelic acid, testosterone and *m*-hydroxybenzoic acid as sole sources of carbon and energy.

Identification of major clusters.

Characters considered to have a presumptive diagnostic value were extracted from Table 14. Some clusters encompassed a wide range of variation so it is not surprising that few unit characters were cluster-specific. Nevertheless there is sufficient consistency within clusters and differences between them for some characters to be weighted for identification. In selecting the distinguishing characters those which were 80% or more positive within a cluster were designated +, those 20% negative or below —, and those with values ranging between 21% and 79% were scored *d* for doubtful. The 64 phenotypic characters most useful for differentiating between clusters are shown in Table 15. It can be seen that some of the tests used for the first time are found to strengthen differentiation between clusters. These include the chitin, deoxyribonucleic acid, guanine and keratin hydrolysis tests, and such nutritional characters as the use of sebacic acid and testosterone as sole sources of carbon and energy. Growth on sugar and sugar alcohols as sole carbon sources were not considered for inclusion in Table 15 because the results of these tests were almost the same as those of the fermentation studies where the same carbohydrates were used.

By using the diagnostic characters all the isolates can be placed in the correct cluster. For

Table 16. *Diagnostic table for the identification of nocardioform organisms*

		<i>Nocardia</i> <i>asteroides</i>	<i>N.</i> <i>caviae</i>	<i>N. bra-</i> <i>siliensis</i>	<i>N. mad-</i> <i>urae</i>	<i>N. pel-</i> <i>letieri</i>	<i>N.</i> <i>turbata</i>	<i>Mycobac-</i> <i>terium</i> <i>rhodoch-</i> <i>rous</i>
Cluster ...		1	2	5	7	8A	10A	14
No. of strains tested ...		42	10	10	22	11	5	104
Acid from:								
28	D-Xylose	—	—	—	+	—	+	—
33	D-Glucose, fermentative	—	—	—	—	—	+	—
Hydrolysis								
Hydrolysis of:								
59	Casein	—	—	+	+	+	+	—
68	Hypoxanthine	—	+	+	+	+	—	—
70	Keratin	—	—	+	+	+	+	—
78	Xanthine	—	+	—	—	—	—	—
Carbon source								
105	Inositol	—	+	+	<i>d</i>	—	—	<i>d</i>
118	Sebacic acid	+	—	+	—	+	—	<i>d</i>
121	Sodium tartrate	—	—	—	—	—	—	+
136	Testosterone	+	+	—	—	—	—	<i>d</i>
202	Resistance to lysozyme	+	+	+	—	—	—	—

Table 17. *Percentage frequency of positive characters found in subgroups of clusters 1 and 2*

Cluster ...	1					2	
Subgroup ...	A	B	C	D	E	A	B
No. of strains tested ...	18	11	6	5	2	4	6
1 Mycelium, formed	100	100	100	100	100	100	100
2 Mycelium, temporary	100	100	100	100	100	100	100
3 Rods and cocci found	100	100	100	100	100	100	100
4 Cocci only	0	0	0	0	0	0	0
5 Rods only	0	0	0	0	0	0	0
6 Acid-fastness, weak	73	30	100	50	100	100	100
7 Acid-fastness, strong	50	20	100	25	100	0	83
8 Strains motile	0	0	0	0	0	0	0
9 Aerial hyphae, sparse	100	90	100	100	100	100	83
10 Aerial hyphae, moderate	100	70	0	75	50	50	50
11 Aerial hyphae, abundant	90	50	0	75	50	0	33
12 Colonies, white/grey	6	0	100	0	0	100	100
13 Colonies, pink/orange/red	90	100	0	25	100	0	0
14 Colonies, black	0	0	0	0	0	0	0
15 Colonies, brown	6	0	0	75	0	0	0
16 Colonies, yellow	0	0	0	0	0	0	0
17 Exopigment, water-soluble	39	70	0	50	50	75	100
18 Colony margin, entire	0	0	0	0	0	0	0
19 Colony margin, filamentous	34	20	100	100	50	100	100
20 Colony margin, deeply filamentous	67	80	0	0	50	0	0
21 Colonies easy to emulsify	0	0	0	0	0	0	50
22 Suspension, coarse	100	100	100	100	100	100	100
23 Colony elevation, flat/raised	0	0	0	0	0	0	0
24 Colony elevation, convex	12	30	0	0	0	0	33
25 Colony elevation, irregular	90	70	100	100	100	100	67

Fermentation studies

Acid from:							
26 L-Arabinose	0	0	0	0	0	0	0
27 D-Arabinose	0	0	0	0	0	0	0
28 D-Xylose	0	0	0	0	0	0	0
29 L-Rhamnose	45	20	0	0	0	0	0
30 D-Fructose	100	90	100	100	100	100	100
31 D-Galactose	22	30	0	25	0	0	0
32 D-Glucose, oxidative	100	90	100	100	100	100	100
33 D-Glucose, fermentative	0	0	0	0	0	0	0
34 Mannose	95	60	100	100	100	100	0
35 Cellobiose	0	10	0	25	0	0	0
36 Lactose	0	0	0	0	0	0	0
37 Sucrose	0	0	0	0	0	0	0
38 Maltose	39	70	34	100	0	25	67
39 Trehalose	39	10	0	50	0	0	83
40 Melezitose	0	0	0	0	0	0	0
41 Raffinose	0	0	0	0	0	0	0
42 Glycogen	0	0	0	0	0	0	0
43 Inulin	0	0	0	0	0	0	0
44 Amygdalin	0	0	0	0	0	0	0
45 Arbutin	95	80	67	75	100	100	100
46 Salicin	12	20	0	50	100	0	0
47 Adonitol	95	0	0	0	0	0	0
48 Dulcitol	0	0	0	0	0	0	0
49 Glycerol	95	90	67	100	100	100	100
50 Mannitol	6	0	34	0	0	100	67
51 Sorbitol	0	6	0	0	0	0	17

Table 17 (*cont.*)

Cluster ...		1					2	
Subgroup ...		A	B	C	D	E	A	B
No. of strains tested ...		18	11	6	5	2	4	6
52	Inositol	0	0	0	0	0	100	100
53	Ethanol	39	0	0	0	50	0	0
54	Dextrin	100	100	67	100	100	100	0
Hydrolysis tests								
55	Adenine, strong	0	0	0	0	0	0	0
56	Adenine, weak	0	0	0	0	0	0	0
57	Aesculin	95	90	100	100	100	100	100
58	Casein, strong	0	0	0	0	0	0	0
59	Casein, weak	0	0	0	0	0	0	0
60	Cellulose	0	0	0	0	0	0	0
61	Chitin	0	0	0	0	0	0	0
62	DNA	6	0	0	50	0	75	0
63	Gelatin, strong	0	0	0	0	0	0	0
64	Gelatin, weak	0	0	0	0	0	0	0
65	Guanine	0	0	0	0	0	75	0
66	Hippurate	0	0	0	0	0	0	0
67	Hypoxanthine, strong	0	0	0	25	50	75	0
68	Hypoxanthine, weak	0	0	0	25	50	100	0
69	Keratin, strong	0	0	0	0	0	0	0
70	Keratin, weak	0	0	0	0	0	0	0
71	Tween 20	100	100	100	100	100	100	83
72	Tween 40	100	100	84	100	100	100	33
73	Tween 60	95	90	34	100	100	100	33
74	Tween 80	12	20	0	0	0	0	50
75	Tyrosine, strong	0	0	0	0	0	0	0
76	Tyrosine, weak	0	0	0	0	0	0	0
77	Xanthine, strong	0	0	0	0	0	75	17
78	Xanthine, weak	0	0	0	0	0	100	100
79	Xylan	0	0	0	0	0	0	0
Sole carbon source								
80	L-Arabinose	0	0	0	0	0	0	0
81	D-Arabinose	0	0	0	0	0	0	0
82	D-Xylose	0	0	0	0	0	0	0
83	L-Rhamnose	61	30	17	50	50	0	0
84	D-Fructose	95	80	100	75	100	100	100
85	D-Galactose	28	30	0	50	0	0	0
86	D-Glucose	100	100	100	100	100	75	100
87	Mannose	90	60	100	100	100	100	0
88	Cellobiose	0	0	0	0	0	75	0
89	Lactose	0	0	0	0	0	0	0
90	Sucrose	12	0	0	0	0	25	17
91	Maltose	61	90	100	50	50	0	100
92	Trehalose	39	10	0	50	0	0	67
93	Melezitose	0	0	0	0	0	0	0
94	Raffinose	0	0	0	0	0	0	0
95	Glycogen	0	0	0	0	0	0	0
96	Inulin	0	0	0	0	0	0	0
97	Amygdalin	12	0	0	0	0	0	0
98	Arbutin	0	0	0	0	0	0	60
99	Salicin	12	20	0	0	0	20	0
100	Adonitol	0	0	0	0	0	0	0
101	Dulcitol	0	0	0	0	0	0	0
102	Glycerol	78	80	17	100	100	100	100

Table 17 (cont.)

Cluster ...	Subgroup ...	1					2	
		A	B	C	D	E	A	B
No. of strains tested ...		18	11	6	5	2	4	6
103	Mannitol	67	30	50	50	50	84	100
104	Sorbitol	12	0	0	25	0	0	17
105	<i>meso</i> -Inositol	0	0	0	0	50	100	83
106	Dextrin	61	50	0	50	100	100	17
107	Starch	67	20	17	25	50	50	67
108	Glucosamine	0	0	0	0	0	0	83
109	Sodium acetate	100	100	100	100	100	100	100
110	Sodium propionate	100	100	100	100	100	50	100
111	Sodium- <i>n</i> -butyrate	100	100	100	100	100	100	100
112	Sodium- <i>n</i> -octoate	0	0	0	0	0	0	0
113	Sodium malonate	0	0	0	0	0	0	0
114	Sodium benzoate	0	0	0	100	0	0	0
115	Sodium succinate	95	90	100	100	100	100	100
116	Adipic acid	73	30	0	100	100	0	0
117	Pimelic acid	0	0	0	0	0	0	0
118	Sebacic acid	95	80	17	100	100	100	100
119	Sodium lactate	0	0	0	0	0	0	0
120	Sodium- <i>H</i> -malate	100	80	100	100	100	100	100
121	Sodium-D-tartrate	0	0	0	0	0	0	0
122	Sodium citrate	6	20	0	50	0	0	83
123	Sodium hippurate	0	0	0	0	0	0	0
124	D- α -Alanine	0	0	0	0	0	0	0
125	L- α -Alanine	0	0	0	0	0	0	60
126	DL-nor-Leucine	0	0	0	0	0	0	0
127	L-Threonine	0	0	0	0	0	0	0
128	L-Serine	0	0	0	0	0	0	83
129	L-Tryptophan	0	0	0	0	0	0	0
130	L-Proline	0	0	0	0	0	0	0
131	L-Tyrosine	0	0	0	0	0	0	0
132	<i>p</i> -Hydroxybenzoic acid	0	0	0	0	0	0	0
133	<i>m</i> -Hydroxybenzoic acid	22	10	0	0	50	0	0
134	D-Mandelic acid	0	0	0	0	0	0	0
135	Protocatechuic acid	12	10	0	0	0	0	0
136	Testosterone	84	60	67	25	100	100	100
137	Sodium pyruvate	100	100	100	100	100	100	100
138	Betaine HCl	0	0	0	0	0	0	0
139	Acetamide	12	0	0	0	0	0	0
140	Benzamide	0	0	0	0	0	0	0
141	<i>p</i> -Cresol	6	0	0	0	0	25	0
142	Paraffin	100	100	100	100	100	75	100
143	Sodium gluconate	0	0	0	0	0	0	0
144	<i>p</i> -Hydroxybenzaldehyde	0	0	0	0	0	25	0
Sole carbon and nitrogen source								
145	Trimethyldiamine	0	0	0	0	0	0	0
146	Serine	0	0	0	0	0	0	0
147	Acetamide	17	0	17	0	100	0	0
Inhibition tests								
Growth on YEA containing:								
148	3 % (w/v) NaCl	100	100	100	100	100	100	100
149	5 % (w/v) NaCl	90	60	84	100	100	100	100
150	7 % (w/v) NaCl	61	30	0	50	100	75	83
151	10 % (w/v) NaCl	0	0	0	0	0	0	0
152	0.001 % (w/v) sodium azide	100	100	100	100	100	100	100

Table 17 (*cont.*)

Cluster ...	Subgroup ...	1					2	
		A	B	C	D	E	A	B
No. of strains tested ...		18	11	6	5	2	4	6
153	0.01 % (w/v) sodium azide	90	20	34	0	100	50	50
154	0.02 % (w/v) sodium azide	22	0	0	0	0	0	0
155	0.03 % (w/v) sodium azide	0	0	0	0	0	0	0
156	0.001 % (w/v) potassium tellurite	100	100	84	100	100	100	100
157	0.01 % (w/v) potassium tellurite	95	70	50	25	100	50	100
158	0.05 % (w/v) potassium tellurite	90	40	67	0	100	83	50
159	0.1 % (w/v) potassium tellurite	84	0	0	0	100	17	0
160	0.1 % (v/v) phenyl ethanol	90	70	100	100	100	100	100
161	0.2 % (v/v) phenyl ethanol	61	30	0	25	100	0	100
162	0.3 % (v/v) phenyl ethanol	39	0	0	0	50	0	87
163	0.4 % (v/v) phenyl ethanol	0	0	0	0	0	0	0
164	0.000001 % (w/v) crystal violet	100	100	100	100	100	100	100
165	0.00001 % (w/v) crystal violet	100	80	100	100	100	100	100
166	0.0001 % (w/v) crystal violet	45	40	50	25	0	25	67
167	0.001 % (w/v) crystal violet	0	0	17	0	0	0	17
168	0.001 % (v/v) Teepol	100	100	100	50	100	100	100
169	0.005 % (v/v) Teepol	84	50	100	25	100	100	100
170	0.01 % (v/v) Teepol	84	20	100	50	100	0	100
171	0.001 % (w/v) phenol	100	100	100	100	100	100	100
172	0.01 % (w/v) phenol	100	60	100	75	100	100	83
173	0.1 % (w/v) phenol	39	0	100	0	50	0	83
174	0.0001 % (w/v) bismuth citrate	95	90	100	100	100	100	100
175	0.001 % (w/v) bismuth citrate	95	70	100	100	100	100	100
176	0.01 % (w/v) bismuth citrate	73	20	0	50	50	75	100
177	0.0001 % (w/v) thallous acetate	96	100	100	100	100	100	100
178	0.001 % (w/v) thallous acetate	6	0	0	0	0	0	33
179	0.01 % (w/v) thallous acetate	0	0	0	0	0	0	0
180	0.01 % (w/v) tetrazolium	67	10	34	50	100	50	100
181	0.01 % (w/v) tetrazolium	0	0	0	0	0	0	0
182	Sensitivity to penicillin	12	70	0	25	0	75	0
Growth at:								
183	pH 5	6	0	0	0	0	0	0
184	pH 6	0	0	84	0	0	0	0
185	pH 8	100	100	100	100	100	100	100
186	pH 9	100	100	84	100	100	100	100
187	pH 10	84	30	83	25	100	100	100
188	50°	50	20	0	0	0	25	0
189	45°	90	40	67	25	100	25	83
190	40°	100	100	100	100	100	75	100
191	35°	100	100	100	100	100	100	100
192	10°	12	60	0	50	0	75	0
Biochemical tests								
193	Benzidine, strong	17	20	17	50	0	50	100
194	Benzidine, weak	100	100	100	100	100	100	100
195	Oxidase	0	20	0	0	0	0	0
196	Catalase	34	50	0	50	0	100	67
197	Nitrate reduction	95	90	100	100	100	100	100
198	Urease, strong	90	90	17	50	100	100	100
199	Urease, weak	95	100	100	100	100	100	100
200	Allantoinase, strong	28	0	0	50	50	25	67
201	Allantoinase, weak	84	64	100	100	100	100	100
202	Lysozyme-resistant	100	100	100	100	100	25	100
203	<i>p</i> -Nitrophenoloxidase	22	0	0	0	50	0	0
204	<i>m</i> -Nitrophenoloxidase	0	0	0	0	0	0	0

instance, strains of *Nocardia asteroides* and *N. brasiliensis* are separated by 17 unit characters. In the past *N. madurae* and *N. pelletieri* strains have been difficult to distinguish, especially if the *N. pelletieri* strains had lost their red pigment. In Table 15 these taxa are separated by 12 species specific characters.

Except with highly atypical or intermediate strains it is neither practical nor necessary to carry out so many tests to achieve accurate identification. A practical diagnostic table was prepared which contained only 11 characters (Table 16). These tests were selected because they gave conclusive results, were easy and quick to perform, and could be carried out even in laboratories with limited resources and equipment. These characters will usually be sufficient for the correct identification of nocardioform organisms which belong to one of the clusters. When necessary the identification of difficult strains can be strengthened by drawing upon additional characters from Table 15.

Identification of subgroups

The percentage frequency with which each character was positive in the subgroups of clusters 1 and 2, *Nocardia asteroides* and *N. caviae* respectively, is shown in Table 17. Although the strains in cluster 1 shared a large number of the properties, particularly negative ones, there were characters which could be used to differentiate the subgroups (Table 18). It is notable that the *N. farcinica* strains could be distinguished from the other subgroups not only by the characters shown in Table 17 but also by their slow growth rate and intense acid-fastness.

Table 18. Distinguishing characters for the subgroups of cluster 1, the *N. asteroides* group

Subgroup ...	A	B	C	D	E
No. of strains tested ...	18	11	6	5	2
11 Aerial hyphae, abundant	+	<i>d</i>	—	+	<i>d</i>
12 Colonies, white/grey	—	—	+	—	—
13 Colonies, orange/pink/red	+	+	—	<i>d</i>	+
15 Colonies, brown	—	—	—	+	—
17 Exopigment, water-soluble	<i>d</i>	<i>d</i>	—	<i>d</i>	<i>d</i>
38 Acid from maltose	<i>d</i>	<i>d</i>	<i>d</i>	+	—
47 Acid from adonitol	+	—	—	—	—
114 Carbon source sodium benzoate	—	—	—	+	—
116 Carbon source adipic acid	<i>d</i>	<i>d</i>	—	+	+
118 Carbon source sebacic acid	+	—	—	+	+
147 Carbon and nitrogen source acetamide	—	—	—	—	+
Inhibition tests, growth on YEA containing:					
150 7% (w/v) NaCl	<i>d</i>	<i>d</i>	—	<i>d</i>	+
153 0.01% (w/v) sodium azide	+	—	<i>d</i>	—	+
158 0.01% (w/v) potassium tellurite	+	—	—	—	+

The subgroups in cluster 2 could also be differentiated by a battery of characters. The *Nocardia caviae* strains form acid from trehalose, but not from mannose, use sodium citrate, glucosamine and L-serine as sole carbon sources and are not inhibited by 0.2% (v/v) phenyl ethanol or 0.1% (w/v) phenol. Alternatively only the *N. uniformis* strains hydrolysed deoxyribonucleic acid, guanine and hypoxanthine.

The distribution of characters to the subgroups of the largest cluster, the *Mycobacterium rhodochrous* cluster, is shown in Table 19. The characters which have a presumptive diagnostic value for differentiating the subgroups are shown in Table 20. Several of these characters were used to construct a practical diagnostic table which can be used for identification of *M. rhodochrous* strains (Table 21).

Table 19. *Percentage frequency of positive characters found in subgroups of cluster 14*

Subgroup ...	A	B	C	D	E	F	G	H
No. of strains tested ...	5	2	19	24	13	7	26	8
1 Mycelium, formed	100	100	100	100	100	0	72	76
2 Mycelium, temporary	100	100	100	100	100	0	72	76
3 Rods and cocci found	100	100	89	100	100	100	78	76
4 Cocci only	0	0	0	0	0	0	12	0
5 Rods only	0	0	11	0	0	0	8	25
6 Acid-fastness, weak	0	0	21	46	0	0	20	0
7 Acid-fastness, strong	0	0	16	17	0	0	12	0
8 Strains motile	0	0	0	0	0	0	0	0
9 Aerial hyphae, sparse	80	0	53	46	23	29	39	13
10 Aerial hyphae, moderate	0	0	0	4	8	0	16	13
11 Aerial hyphae, abundant	0	0	0	0	0	0	8	0
12 Colonies, white/grey	0	0	0	59	23	57	43	63
13 Colonies, pink/orange/red	100	100	100	42	77	28	51	0
14 Colonies, black	0	0	0	0	0	0	0	0
15 Colonies, brown	0	0	0	0	0	0	0	0
16 Colonies, yellow	0	0	0	0	0	14	8	38
17 Exopigment, water soluble	0	0	11	4	15	0	8	13
18 Colony margin, entire	0	0	79	100	85	100	62	38
19 Colony margin, filamentous	100	100	21	0	15	0	27	25
20 Colony margin, deeply filamentous	0	0	0	0	0	0	12	38
21 Colonies easy to emulsify	100	100	100	100	100	100	59	100
22 Suspension, coarse	0	0	0	0	0	0	55	0
23 Colony elevation, flat/raised	80	0	0	17	23	0	0	13
24 Colony elevation, convex	20	100	63	71	54	57	72	76
25 Colony elevation, irregular	0	0	37	13	23	43	27	13

Fermentation studies

Acid from:

26 L-Arabinose	0	0	0	0	15	0	12	63
27 D-Arabinose	0	0	0	0	0	0	0	0
28 D-Xylose	0	0	0	0	0	0	8	38
29 L-Rhamnose	0	0	0	0	8	0	4	0
30 D-Fructose	100	100	100	100	100	100	66	110
31 D-Galactose	0	0	0	4	22	0	23	63
32 D-Glucose, oxidative	100	100	100	100	100	100	70	100
33 D-Glucose, fermentative	0	0	0	0	0	0	0	0
34 Mannose	100	100	100	92	85	100	55	100
35 Cellobiose	100	0	0	0	0	0	4	63
36 Lactose	0	0	0	0	8	0	4	38
37 Sucrose	100	100	80	92	100	14	35	100
38 Maltose	100	100	100	100	92	14	47	88
39 Trehalose	100	100	90	92	77	29	31	50
40 Melezitose	0	0	0	0	0	0	4	38
41 Raffinose	0	0	0	0	0	0	8	38
42 Glycogen	0	0	0	0	0	0	0	63
43 Inulin	0	0	0	0	0	0	0	0
44 Amygdalin	0	0	0	0	15	0	16	38
45 Arbutin	0	5	37	71	31	0	56	88
46 Salicin	0	50	48	50	39	0	12	38
47 Adonitol	0	0	0	0	23	0	0	0
48 Dulcitol	0	0	0	0	0	0	0	0
49 Glycerol	100	100	100	100	92	100	12	63
50 Mannitol	100	100	100	100	92	100	16	50
51 Sorbitol	100	100	100	100	100	72	8	13
52 meso-Inositol	0	0	0	38	23	14	20	0
53 Ethanol	100	100	100	100	69	14	20	13
54 Dextrin	100	100	100	100	76	29	59	100

Table 19 (cont.)

Subgroup ...	A	B	C	D	E	F	G	H
No. of strains tested ...	5	2	19	24	13	7	26	8
Hydrolysis tests								
55 Adenine, strong	0	0	53	67	54	0	16	0
56 Adenine, weak	100	100	69	71	62	0	20	0
57 Aesculin	100	100	95	100	92	100	84	88
58 Casein, strong	0	0	0	0	8	15	8	100
59 Casein, weak	0	0	0	0	8	57	8	100
60 Cellulose	0	0	0	0	0	0	0	0
61 Chitin	0	0	0	0	0	0	0	0
62 DNA	0	100	42	92	69	86	50	88
63 Gelatin, strong	0	0	0	0	0	86	16	63
64 Gelatin, weak	0	0	0	0	0	86	39	100
65 Guanine	0	0	0	0	23	100	4	0
66 Hippurate	0	0	5	0	0	0	0	0
67 Hypoxanthine, strong	0	0	0	0	8	14	0	0
68 Hypoxanthine, weak	0	0	0	0	8	14	4	0
69 Keratin, strong	0	0	0	0	0	0	0	25
70 Keratin, weak	0	0	0	0	0	0	13	38
71 Tween 20	100	100	100	100	100	100	90	100
72 Tween 40	100	100	100	100	100	100	74	100
73 Tween 60	100	100	100	100	100	100	74	76
74 Tween 80	100	0	21	4	0	0	8	13
75 Tyrosine, strong	100	50	100	38	46	57	16	0
76 Tyrosine, weak	100	50	100	59	46	72	16	0
77 Xanthine, strong	0	0	0	0	8	14	0	0
78 Xanthine, weak	0	0	0	0	8	14	4	0
79 Xylan	0	0	0	0	0	0	0	0
Sole carbon source								
80 L-Arabinose	0	100	85	71	39	14	4	100
81 D-Arabinose	0	0	0	0	0	0	0	13
82 D-Xylose	100	100	74	63	54	0	35	100
83 L-Rhamnose	0	0	0	0	8	0	20	0
84 D-Fructose	20	100	100	92	92	100	55	100
85 D-Galactose	0	100	64	25	46	57	31	88
86 D-Glucose	100	100	95	100	100	100	82	100
87 Mannose	100	100	95	96	85	100	74	100
88 Cellobiose	100	0	16	4	15	0	12	100
89 Lactose	100	0	0	0	15	29	12	50
90 Sucrose	100	100	85	76	77	14	50	100
91 Maltose	100	0	58	59	77	57	43	88
92 Trehalose	100	100	100	100	92	29	12	100
93 Melezitose	100	0	0	0	15	0	16	63
94 Raffinose	100	0	0	0	8	29	4	38
95 Glycogen	0	0	0	0	0	0	4	76
96 Inulin	100	0	0	0	15	0	8	0
97 Amygdalin	0	0	0	0	0	0	4	38
98 Arbutin	0	0	0	4	0	0	16	0
99 Salicin	0	100	53	59	23	0	25	38
100 Adonitol	0	0	0	0	23	0	0	0
101 Dulcitol	0	0	0	0	0	0	20	25
102 Glycerol	100	50	100	92	100	100	39	88
103 Mannitol	80	100	100	96	77	43	31	76
104 Sorbitol	80	100	100	96	77	0	12	38
105 meso-Inositol	0	0	0	63	46	0	4	0
106 Dextrin	60	50	69	50	15	0	8	38
107 Starch	100	100	58	46	23	0	39	88

Table 19 (*cont.*)

Subgroup ...	A	B	C	D	E	F	G	H
No. of strains tested ...	5	2	19	24	13	7	26	8
108 Glucosamine	0	0	48	84	54	0	4	90
109 Sodium acetate	0	100	100	92	92	72	46	100
110 Sodium propionate	100	100	100	100	100	100	74	38
111 Sodium- <i>n</i> -butyrate	100	100	100	92	100	100	60	4
112 Sodium- <i>n</i> -octoate	100	0	80	34	23	0	23	0
113 Sodium malonate	0	0	0	0	0	0	0	0
114 Sodium benzoate	0	50	80	13	23	100	0	0
115 Sodium succinate	100	100	95	100	85	72	23	0
116 Adipic acid	100	50	100	88	70	0	12	0
117 Pimelic acid	0	50	100	63	46	14	8	0
118 Sebacic acid	0	100	100	92	62	0	20	0
119 Sodium lactate	100	100	100	88	39	0	8	38
120 Sodium- <i>H</i> -malate	0	100	100	100	92	86	35	50
121 Sodium- <i>D</i> -tartrate	100	0	0	4	15	14	0	13
122 Sodium citrate	100	100	100	88	77	100	16	38
123 Sodium hippurate	0	0	0	0	8	0	4	0
124 D- α -Alanine	0	100	64	59	85	0	12	13
125 L- α -Alanine	0	100	16	92	85	0	27	13
126 DL-nor-leucine	100	50	90	42	39	0	0	0
127 L-Threonine	0	0	0	8	31	14	4	13
128 L-Serine	0	50	5	59	70	14	8	0
129 L-Tryptophan	0	0	0	0	0	0	0	0
130 L-Proline	0	50	48	50	46	0	16	13
131 L-Tyrosine	100	50	100	17	23	0	0	13
132 <i>p</i> -Hydroxybenzoic acid	0	100	80	92	39	0	4	0
133 <i>m</i> -Hydroxybenzoic acid	100	100	80	4	8	0	35	0
134 D-Mandelic acid	0	0	0	0	0	0	4	0
135 Protocatechuic acid	100	100	100	84	62	28	20	25
136 Testosterone	100	0	100	96	69	0	23	0
137 Sodium pyruvate	100	100	100	100	100	100	59	38
138 Betaine HCl	0	0	0	0	0	0	8	0
139 Acetamide	100	100	95	88	77	29	4	25
140 Benzamide	100	0	16	0	0	0	0	0
141 <i>p</i> -Cresol	100	100	100	29	0	0	4	0
142 Paraffin	100	100	100	96	92	100	59	76
143 Sodium gluconate	0	100	80	96	92	43	23	76
144 <i>o</i> -Hydroxybenzaldehyde	100	50	37	21	39	0	4	0

Sole carbon and nitrogen source

145 Trimethyldiamine	0	50	42	4	0	0	0	0
146 Serine	0	0	0	0	0	0	8	0
147 Acetamide	0	0	0	0	0	0	0	0

Inhibition tests

Growth on YEA containing:								
148 3% (w/v) NaCl	100	100	100	100	100	100	78	100
149 5% (w/v) NaCl	100	0	100	92	100	100	50	88
150 7% (w/v) NaCl	100	0	85	80	85	86	47	50
151 10% (w/v) NaCl	80	0	5	0	15	57	4	13
152 0.001% (w/v) sodium azide	100	100	100	100	100	100	100	100
153 0.01% (w/v) sodium azide	0	0	80	100	85	43	39	76
154 0.02% (w/v) sodium azide	0	0	42	84	77	14	20	38
155 0.03% (w/v) sodium azide	0	0	5	71	39	14	8	25
156 0.001% (w/v) potassium tellurite	100	100	100	100	100	100	82	100
157 0.01% (w/v) potassium tellurite	100	100	100	84	100	100	70	88
158 0.05% (w/v) potassium tellurite	100	0	100	63	92	57	31	25
159 0.1% (w/v) potassium tellurite	100	0	74	8	77	29	28	0
160 0.1% (v/v) phenyl ethanol	100	100	100	100	100	100	96	100

Table 19 (cont.)

Subgroup ...	A	B	C	D	E	F	G	H
No. of strains tested ...	5	2	19	24	13	7	26	8
161 0.2 % (v/v) phenyl ethanol	100	100	100	100	100	86	62	100
162 0.3 % (v/v) phenyl ethanol	100	0	100	96	85	29	47	88
163 0.4 % (v/v) phenyl ethanol	0	0	11	58	31	0	35	25
164 0.000001 % (w/v) crystal violet	100	100	100	100	100	100	66	100
165 0.00001 % (w/v) crystal violet	100	100	100	100	100	100	55	100
166 0.0001 % (w/v) crystal violet	100	50	80	46	54	57	23	25
167 0.001 % (w/v) crystal violet	0	0	42	0	15	0	8	0
168 0.001 % (v/v) Teepol	100	100	100	100	100	100	100	100
169 0.005 % (v/v) Teepol	100	100	100	100	100	100	62	88
170 0.01 % (v/v) Teepol	100	100	90	100	100	14	43	88
171 0.001 % (w/v) phenol	100	100	100	100	100	100	100	100
172 0.01 % (w/v) phenol	100	100	100	100	100	57	59	100
173 0.1 % (w/v) phenol	100	100	80	55	46	0	8	63
174 0.0001 % (w/v) bismuth citrate	100	100	100	100	100	100	82	100
175 0.001 % (w/v) bismuth citrate	100	100	100	100	100	57	50	88
176 0.01 % (w/v) bismuth citrate	100	100	58	29	62	0	8	76
177 0.0001 % (w/v) thallous acetate	100	100	100	100	100	100	66	100
178 0.001 % (w/v) thallous acetate	0	50	16	4	15	100	16	38
179 0.01 % (w/v) thallous acetate	0	0	0	0	0	0	8	0
180 0.01 % (w/v) tetrazolium	100	100	100	100	92	100	82	100
181 0.1 % (w/v) tetrazolium	0	0	0	0	0	0	4	13
182 Sensitivity to penicillin	100	100	100	100	100	100	78	88
Growth at:								
183 pH 5	0	0	0	8	0	0	0	0
184 pH 6	100	100	100	100	100	100	86	76
185 pH 8	100	100	100	100	100	100	86	88
186 pH 9	100	100	100	84	92	100	62	76
187 pH 10	100	100	100	84	62	86	39	38
188 50°	0	0	0	4	0	0	0	0
189 45°	0	0	11	4	8	0	20	0
190 40°	100	50	100	92	92	0	62	63
191 35°	100	100	100	96	100	100	66	88
192 10°	100	100	100	84	92	100	22	76
Biochemical tests								
193 Benzidine, strong	100	100	100	100	100	100	100	25
194 Benzidine, weak	100	100	100	100	100	100	100	100
195 Oxidase	20	0	0	0	0	29	4	0
196 Catalase	100	100	100	100	77	86	59	25
197 Nitrate reduction	100	100	100	88	92	86	70	88
198 Urease, strong	0	50	0	84	85	14	39	25
199 Urease, weak	0	100	0	84	92	14	59	25
200 Allantoinase, strong	0	0	0	4	23	14	8	0
201 Allantoinase, weak	0	100	0	59	85	14	27	25
202 Lysozyme resistant	0	0	0	0	0	0	12	0
203 <i>p</i> -Nitrophenoloxidase	100	0	0	0	8	14	0	0
204 <i>m</i> -Nitrophenoloxidase	0	50	42	4	0	0	0	0

CONCLUSIONS

The test strains were recovered in a few defined clusters. Jones & Bradley (1964) and Cerbón (1967) in their numerical taxonomic studies did not obtain well-defined groups, although this was probably due to inadequate strain selection. Tsukamura (1969) examined more strains, which clustered well, but many of the unit characters he used were ineffective for differentiating nocardiae, and recent studies by P. V. Kurup & J. A. Schmitt (personal

Table 20. *Distinguishing characters for the subgroups of cluster 14, the Mycobacterium rhodochrous group*

Subgroup ...	A	B	C	D	E	F	G	H
No. of strains tested ...	5	2	19	24	13	7	26	8
1 Mycelium, formed	+	+	+	+	+	—	<i>d</i>	<i>d</i>
13 Colonies, pink/orange/red	+	+	+	<i>d</i>	+	<i>d</i>	<i>d</i>	—
19 Colony margin, filamentous	+	+	—	—	—	—	<i>d</i>	<i>d</i>
Acid from:								
35 Cellobiose	+	—	—	—	—	—	—	<i>d</i>
37 Sucrose	+	+	+	+	+	—	<i>d</i>	+
49 Glycerol	+	+	+	+	+	+	—	<i>d</i>
51 Sorbitol	+	+	+	+	+	<i>d</i>	—	—
Hydrolysis of:								
56 Adenine, weak	+	+	<i>d</i>	<i>d</i>	<i>d</i>	—	—	—
58 Casein, strong	—	—	—	—	—	—	—	+
62 DNA	—	+	<i>d</i>	+	<i>d</i>	+	<i>d</i>	+
65 Guanine	—	—	—	—	<i>d</i>	+	—	—
74 Tween 80	+	—	—	—	—	—	—	—
75 Tyrosine, strong	+	<i>d</i>	+	<i>d</i>	<i>d</i>	<i>d</i>	—	—
Sole carbon source:								
108 Glucosamine	—	—	<i>d</i>	+	<i>d</i>	—	—	—
109 Sodium acetate	—	+	+	+	+	<i>d</i>	<i>d</i>	+
112 Sodium- <i>n</i> -octoate	+	—	+	<i>d</i>	<i>d</i>	—	<i>d</i>	—
114 Sodium benzoate	—	<i>d</i>	+	—	<i>d</i>	+	—	—
116 Adipic acid	+	<i>d</i>	+	+	<i>d</i>	—	—	—
117 Pimelic acid	—	<i>d</i>	+	<i>d</i>	<i>d</i>	—	—	—
118 Sebacic acid	—	+	+	+	<i>d</i>	—	—	—
119 Sodium lactate	+	+	+	+	<i>d</i>	—	—	<i>d</i>
120 Sodium- <i>H</i> -malate	—	+	+	+	+	+	<i>d</i>	<i>d</i>
121 Sodium- <i>D</i> -tartrate	—	—	—	—	—	—	—	—
125 L- α -Alanine	—	+	—	+	+	—	<i>d</i>	—
126 DL-nor-Leucine	+	<i>d</i>	+	<i>d</i>	<i>d</i>	—	—	—
131 L-Tyrosine	+	<i>d</i>	+	—	<i>d</i>	—	—	—
132 <i>p</i> -Hydroxybenzoic acid	—	+	+	+	<i>d</i>	—	—	—
133 <i>m</i> -Hydroxybenzoic acid	+	+	+	—	—	—	<i>d</i>	—
136 Testosterone	+	—	+	+	<i>d</i>	—	<i>d</i>	—
140 Benzamide	+	—	—	—	—	—	—	—
141 <i>p</i> -Cresol	+	+	+	<i>d</i>	—	—	—	—
144 <i>o</i> -Hydroxybenzaldehyde	+	<i>d</i>	<i>d</i>	—	<i>d</i>	—	—	—
Inhibited by:								
150 7% (w/v) NaCl	+	—	+	+	+	+	<i>d</i>	<i>d</i>
153 0.01% (w/v) sodium azide	—	—	+	+	+	<i>d</i>	<i>d</i>	<i>d</i>
162 0.3% (v/v) phenyl ethanol	+	—	+	+	+	<i>d</i>	<i>d</i>	+
Biochemical tests								
199 Urease, weak	—	+	—	+	+	—	<i>d</i>	<i>d</i>
201 Allantoinase, weak	—	+	—	<i>d</i>	+	—	<i>d</i>	<i>d</i>
203 <i>p</i> -Nitrophenoloxidase	+	—	—	—	—	—	—	—

communication, 1971) have included only the strongly mycelial or 'hard' growing nocardiae. The distribution of nocardioform bacteria to 15 distinct clusters was, therefore, encouraging.

The five well-known pathogenic species of *Nocardia* were recovered in and characterize five of the major clusters. Cluster 1 contained *Nocardia asteroides* strains and cultures bearing nine specific names already reduced to synonyms of that species (Gordon & Mihm, 1957, 1959*a*, 1962*b*). With the exception of *N. farcinica* these nomenspecies are undoubtedly

Table 21. *Diagnostic table for the identification for the subgroups of cluster 14, the Mycobacterium rhodochrous group*

Subgroup ...	A	B	C	D	E	F	G	H
No. of strains tested ...	5	2	19	24	13	7	26	8
I Mycelium, formed	+	+	+	+	+	—	<i>d</i>	<i>d</i>
35 Acid from Cellobiose	+	—	—	—	—	—	—	<i>d</i>
Hydrolysis of:								
56 Adenine, weak	+	+	<i>d</i>	<i>d</i>	<i>d</i>	—	—	—
58 Casein, strong	—	—	—	—	—	—	—	+
74 Tween 80	+	—	—	—	—	—	—	—
Sole carbon source:								
114 Sodium benzoate	—	<i>d</i>	+	—	<i>d</i>	+	—	—
118 Sebacic acid	—	+	+	+	<i>d</i>	—	—	—
132 <i>p</i> -Hydroxybenzoic acid	—	+	+	+	<i>d</i>	—	—	—
136 Testosterone	+	—	+	+	<i>d</i>	—	<i>d</i>	—
141 <i>p</i> -cresol	+	+	+	<i>d</i>	—	—	—	—
Biochemical tests								
199 Urease, weak	—	+	—	+	+	—	<i>d</i>	<i>d</i>
203 <i>p</i> -Nitrophenoloxidase	+	—	—	—	—	—	—	—

historical legacies based upon a few strains which were inadequately characterized. The strains in the *N. asteroides* cluster exhibited a common set of characters but also showed a high degree of variability under the test conditions. *Nocardia asteroides* was also found to be heterogeneous in work based upon morphological and biochemical criteria (Georg, Ajello, McDermont & Hosty, 1961; Cerbón, 1967), on physiological characters (Mariat, 1965), and on serological properties (Kwapinski, 1970; Pier & Fichtner, 1971). P. V. Kurup & J. A. Schmitt (personal communication, 1971) divided *N. asteroides* into four subgroups, whereas Tsukamura (1969) divided it into two major clusters one of which he designated *N. farcinica*. The results of the present study confirm that *N. asteroides* can be divided into homogeneous subgroups which can be defined and identified by characters extracted from the data. Further work is required to throw more light on the taxonomic status of these subgroups.

Subgroup I C is of particular interest for it contains the type species of the genus, including what may be the original type strain, *Nocardia farcinica*, ATCC 3318. The other strains in this subgroup were isolated from cases of bovine farcy in West Africa. Gordon & Mihm (1962*b*) considered *N. farcinica* to be a synonym of *N. asteroides*, but Mariat (1963) and Magnusson & Mariat (1968) considered that *N. farcinica* should be given independent specific status. The present results and those of Tsukamura (1969) confirm that *N. farcinica* strains fall into a group which can be separated from those containing *N. asteroides* strains. Subgroup I C could not, however, be directly equated with Tsukamura's *N. farcinica* cluster. The respective groups did contain strain ATCC 3318, but several bacteria considered to be *N. farcinica* by Tsukamura (1969) were found in subgroups I A and I C. Tsukamura (1969) did not study any of the slow-growing, strongly acid-fast *N. farcinica* from West Africa, bacteria which have been considered to be mycobacteria because they contain mycolic acids (Chamoiseau & Asselineau, 1970; Lechevalier *et al.* 1971). It seems probable that *N. farcinica* strains from West Africa are different from other strains bearing this name. If so it is puzzling that strain ATCC 3318, allegedly Nocard's original isolate, shares such a high general similarity with the slow growing *N. farcinica* strains from West Africa. It may be that in this case cluster formation has been influenced by factors such as growth rate. Recently Sneath (1968) has described a method of calculating phenetic similarities which takes into account differences which can be

explained by factors such as the unequal growth rates of strains. This programme will be applied to the test data and the results will show whether cluster formation in subgroup 1C has been affected by growth rate.

The *Nocardia caviae* and *N. brasiliensis* strains fell into tight clusters, a result which is in good agreement with those of Tsukamura (1969). The distinction between each of these species, and between them and *N. asteroides*, is maintained. Additional work is required to see whether *N. uniformis* should be considered as a synonym of *N. caviae* or whether it forms a distinct group lying between *N. caviae* and *N. asteroides*. The *N. asteroides*, *N. caviae* and *N. brasiliensis* clusters unite to form an aggregate group, sharply differentiated from the other major clusters at the 75% S-level. These three species, therefore, share a high phenetic similarity, have a wall composition of type IV (Lechevalier, Lechevalier & Becker, 1966), contain a characteristic lipid (Mordarska & Mordarski, 1969) and have strong serological affinities (Kwapinski, 1970). The three clusters form the core of the genus *Nocardia*, and *N. caviae* and *N. brasiliensis* can be considered to be taxospecies.

The *Nocardia madurae* and *N. pelletieri* strains are found in two major clusters which join the nocardia clusters only at a low level of similarity. These species have been transferred to the genus *Streptomyces* (Mackinnon & Artagaveytia-Allende, 1956; Mariat, 1958, 1962) but Gordon (1966a) retained them as *Nocardia*. *Nocardia pelletieri* and *N. madurae* strains can be separated from *N. asteroides*, *N. caviae* and *N. brasiliensis* on the phenetic evidence, serologically (Kwapinski, 1970), and they have a wall composition of type III (Lechevalier *et al.* 1966). They share a low phenetic similarity with *Streptomyces* spp. which have a wall composition of type I (Lechevalier *et al.* 1966). The results of the numerical taxonomic study therefore lend strong support to the proposal that *N. madurae* and *N. pelletieri* be placed in the new genus *Actinomadura* (Lechevalier & Lechevalier, 1970). This genus was defined exclusively on morphological and chemotaxonomic evidence and many of these characters are difficult to detect. In the present work it was possible to distinguish the *N. madurae* and *N. pelletieri* clusters from one another, and from the other nocardioform clusters by a battery of repeatable characters which were easy to observe.

The phenetic similarity between *Actinomadura madurae* and *A. pelletieri* is not high. Since *A. pelletieri* strains grew very slowly the low similarity may be a function of growth rates and not be due to a large difference in the properties of the two species. The application of the vigour and pattern programme (Sneath, 1968) will show whether this is so. It is interesting to find that the *A. madurae* cluster is heterogeneous. Further taxonomic work is needed on *A. madurae* strains; perhaps strains from different habitats fall into distinct groups. Lechevalier & Lechevalier (1970) included *Nocardia dassonvillei* in the genus *Actinomadura*. This proposal cannot be strongly supported by the present results because the *N. dassonvillei* cluster is sharply separated from all the other clusters although it is true that the highest similarity shown by the *N. dassonvillei* strains is with *A. madurae* strains.

The *Nocardia turbata* strains share a high phenetic similarity and are quite distinct from the other clusters. Sukapure *et al.* (1970) found that *turbata* strains did not fit into any previously described genera. Their inclusion in the new genus *Oerskovia* (Prauser *et al.* 1970) is strongly supported.

In my preliminary studies *Mycobacterium rhodochrous* strains formed a distinct cluster readily delineated from other clusters containing nocardioform bacteria. The *M. rhodochrous* cluster is recovered in this enlarged study and contains strains carrying over 30 specific names. Gordon & Mihm (1959b, 1961) and Gordon (1966b) also found that the alleged differences between species of 'soft' growing nocardioform strains were tenuous and reduced many of the names to synonyms of *M. rhodochrous*. Additional species continue to be identi-

fiable with *M. rhodochrous* (Mitchell & Shewan, 1968) and *Nocardia pellegrino* can now be added to them. Castelnova, Bellezza, Duncan & Asselineau (1964) and Juhasz & Bönicke (1965) also placed *N. pellegrino* strains into the same group as *Nocardia corallina*, *N. rubra* and certain *M. rhodochrous* strains. The *M. rhodochrous* cluster is heterogeneous and can be divided into subgroups which can be defined and identified by a group of characters. There is no reliable way of equating clusters or subgroups with orthodox taxonomic ranks. Numerical taxonomists generally adjust the level of similarity to meet their own concept of species. Further work is required to find out whether the homogeneous subgroups – 14A which contains *N. pellegrino*, 14C which includes *N. corallina*, *N. rubra* and *N. salmonicolor*, 14D which includes *N. erythropolis*, *N. rubropertincta* and *Jensenia canicruria*, and 14F which contains arthrobacter and corynebacteria strains – can be equated with taxospecies or biotypes. In this context it is interesting that Adams, Adams & Brownell (1970) considered *J. canicruria* to be a synonym of *N. erythropolis* but different from *M. rhodochrous* strains.

Although the *Mycobacterium rhodochrous* group constitutes a recognizable taxonomic entity its generic location is still unsettled. *Mycobacterium rhodochrous* strains show a closer phenetic relationship with *Nocardia asteroides*, *N. caviae* and *N. brasiliensis* than with strains of Runyon's group IV mycobacteria. Recently Lechevalier *et al.* (1971) have found that *M. rhodochrous* strains contain nocardomycolic acids but the fast growing mycobacteria show the presence of mycolic acids. It would appear that the *M. rhodochrous* group shows a greater taxonomic affinity to *Nocardia* than to *Mycobacterium* and could be provisionally placed in the genus *Nocardia*. Further work is needed to see if the rhodochrous group deserves recognition as a genus, and to establish the relationship of the taxon with diphtheroid bacteria, particularly arthrobacter.

The taxonomic status of the minor clusters and of the unclustered strains is not clear. Some of these bacteria may be nuclei around which future species of *Nocardia* will cluster, others are strains which are misplaced in the genus. The former include the minor clusters containing *Nocardia gardneri* and *N. vaccinii*, and the strains of *N. hydrocarboxydans*, *N. narashinoensis* and *N. petroleophila*. Alternatively the *N. formica*, *N. italica* and *S. somaliensis* strains are phenetically distinct, contain major amounts of L-2,6-diaminopimelic acid, and should be placed in the genus *Streptomyces*. The taxonomic position of strains such as *N. coeliaca*, *N. lurida*, *N. rugosa*, *N. saturnea* and *Cellulomonas biazotea* is still confused.

One of the advantages of numerical taxonomy is that the clusters which are defined have a high information content. Each cluster can be examined for discriminating features that can be weighted for identification. In the present study it was possible to construct a diagnostic table for the identification of the main clusters of nocardioform bacteria. In the case of the *Nocardia asteroides* and *Mycobacterium rhodochrous* clusters tables were devised for the identification of the subgroups. Several features are needed to define each cluster or subgroup, so that no one character is either sufficient or necessary to identify an isolate. The clusters are therefore operationally polythetic and can accommodate a degree of strain variability thereby reducing the chances of variants of a species being defined as new species. Classical tests such as acid-fastness, pigmentation and paraffin utilization were found not to have a high differentiating power, a result which emphasizes the danger of creating taxa on the basis of a few subjectively chosen characters. Alternatively some of the tests which had not been performed before did have a high diagnostic value. They include the utilization of some of the carbon compounds as sole sources of carbon and energy, and keratin and deoxyribonucleic acid hydrolysis. Although many of the carbon compounds did not support growth their failure to do so is open to several interpretations, such as complexing of the compounds in the medium or because the strains were sensitive to the toxic effects of the

compounds at the concentrations used, or because the bacteria were unable to synthesize the enzymes necessary for metabolizing the compounds. It was noted that the results of some of the tests did not agree with those obtained by Tsukamura (1969). Now that significant groups of nocardioform bacteria have been obtained the search for additional simple reproducible phenotypic characters which can be weighted for identification can be intensified.

In conclusion, it can be said that the results confirm that the earlier taxonomy of the nocardioform bacteria was illogical. They also show that most of the nocardioform bacteria fall into one or other of several large recognizable groups and it is hoped that additional isolates will be allocated to the correct group by using the relevant diagnostic table. It should be stressed, however, that the results of this study, which are useful and many, represent only a first step towards characterizing the phenotypes of nocardioform bacteria. Future studies can, for the first time, be carried out on strains which have a recognized taxonomic identity. The genus *Nocardia* can no longer be considered a taxon of convenience.

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