Numerical Classification of "*Mycobacterium*" *rhodochrous* and Runyon's Group IV *Mycobacteria*

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A numerical classification study was carried out on 129 strains representing "*Mycobacterium*" *rhodochrous*, Runyon's Group IV mycobacteria, and other related taxa. Cultures of these strains were examined for 170 characters, and the resultant data have been analyzed by computer. Similarity coefficients were calculated in two ways: by the first, negative and positive matches were scored, and in the second, positive similarities only; in each case, clustering was with the average linkage cluster analysis technique. The two coefficients of association yielded the same four clusters, and, with a single exception, substantially the same subgroups. The three homogeneous clusters corresponded to: (i) "*M.*" *rhodochrous*, (ii) *Nocardia pellegrino*, and (iii) *Mycobacterium* Group IV. Twenty-three named mycobacteria were recovered in seven subgroups, six of which were equated with the established taxa *M. borstelense*, *M. chitae*, *M. gallinarum*, *M. fortuitum*, *M. smegmatis*, and *M. vaccae*. Characters were abstracted from the data and used for the identification of clusters and subgroups.

The generic location of "*Mycobacterium*" *rhodochrous* has always been uncertain (8, 9). Gordon (7) considered the properties of this taxon to be intermediate between those of *Nocardia* and *Mycobacterium* but different from *Corynebacterium*. The results of an extensive numerical taxonomic study, however, have shown that "*M.*" *rhodochrous* strains form a taxon which occupies a similar rank to clusters which contain nocardiae, actinomadurae, and oerskoviae (6). Since no corresponding large-scale study had been done comparing rapidly growing mycobacteria and "*M.*" *rhodochrous*, this name was retained for convenience, but its temporary nature was recognized. This taxon is included in the newly proposed genus *Gordona* (27) but only on the basis of evidence collected from six named strains. *Jensenia canicruria* is now considered to belong to the "*M.*" *rhodochrous* group (6), so that if this taxon is to be a genus, *Gordona* is a later synonym of the earlier name *Jensenia*. The primary aim of this study was to compare representative strains of fast-growing mycobacteria with strains drawn from the "*M.*" *rhodochrous* complex.

Many new species have been described since Runyon classified atypical mycobacteria into four groups (17). New species have been proposed particularly commonly in Group IV (1-3, 16, 23-25). Although some comparative taxonomic work has been done (11, 12, 24, 28), it is by no means clear whether the differences between some of these new taxa merit their separation into species. A second purpose of this paper was to establish the quantitative relationships between the species of fast-growing mycobacteria.

All the test organisms were examined for characters which had been included in a previous analysis (6). Although the test selection was intended to be unbiased, it was not known whether characters which had been found useful in the classification of nocardioform bacteria would be relevant in studying mycobacteria. It has been shown that a large number of inappropriate tests can introduce levels of "noise" sufficient to obscure differences between taxa (5). To ensure against such a possibility, the results of a single coefficient of association should not always be accepted uncritically. In the present study, similarity

TABLE 1. *Strains examined*

Strain	Laboratory no. ^a	Source	Comment
M29 <i>Mycobacterium abscessus</i>	LA 948	ATCC 19977	Type strain
M88 <i>M. abscessus</i>	ATCC 19977	ATCC 19977	Type strain
M12 <i>M. aurum</i>	NCTC 10437	ATCC 23366	Type strain; Biotype 1
M13 <i>M. aurum</i>	NCTC 10438	NCTC 10438	Biotype 1
M15 <i>M. aurum</i>	NCTC 10440	NCTC 10440	Biotype 11
M155 <i>M. borstelense</i>	R. Bönicke, SN 281 ^b	ATCC 19235	Type strain
M90 <i>M. borstelense</i> subsp. <i>niacinogenes</i>	ATCC 19237	R. Bönicke SN 286	Type strain
M156 <i>M. borstelense</i> subsp. <i>niacinogenes</i>	R. Bönicke SN 286	ATCC 19237	Type strain
M157 <i>M. borstelense</i> subsp. <i>niacinogenes</i>	R. Bönicke SN 284	ATCC 19537	
M91 <i>M. butyricum</i>	ATCC 19979	ATCC 19979	
M25 <i>M. chitae</i>	NCTC 10485	ATCC 19627	Type strain
M26 <i>M. chitae</i>	NCTC 10495	NCTC 10495	
M73 <i>M. chitae</i>	M. Tsukamura ^c 2050	ATCC 19629	
M74 <i>M. chitae</i>	M. Tsukamura 2050	ATCC 19630	
M92 <i>M. diernhoferi</i>	ATCC 19340	R. Bönicke SN 1418	Type strain
M158 <i>M. diernhoferi</i>	R. Bönicke SN 1418	ATCC 19340	Type strain
M159 <i>M. diernhoferi</i>	R. Bönicke SN 1403	R. Bönicke SN 1043	
M160 <i>M. diernhoferi</i>	R. Bönicke SN 1425	ATCC 19344	
M61 <i>M. fortuitum</i>	I. Baess ^d F 656		Froman, 70 <i>M. minetti</i>)
M62 <i>M. fortuitum</i>	I. Baess F 141	ATCC 9820	
M93 <i>M. fortuitum</i>	ATCC 6841		
M79 <i>M. gallinarum</i>	M. Tsukamura 2506	ATCC 19710	Type strain
M80 <i>M. gallinarum</i>	M. Tsukamura 2507	ATCC 19711	
M38 <i>M. giae</i>	LA 82	ATCC 11440	
M94 <i>M. lacticola</i>	ATCC 19980		
M95 <i>M. minetti</i>	ATCC 19542		
M16 <i>M. parafortuitum</i>	NCTC 10410		
M17 <i>M. parafortuitum</i>	NCTC 10411	ATCC 19686	Type strain
M53 <i>M. parafortuitum</i>	LA 2488	ATCC 19688	
M77 <i>M. parafortuitum</i>	M. Tsukamura 305		
M78 <i>M. parafortuitum</i>	M. Tsukamura 2590		
M96 <i>M. parafortuitum</i>	ATCC 19686		Type strain
M97 <i>M. parafortuitum</i>	ATCC 19687		
M6 <i>M. peregrinum</i>	NCTC 10264	ATCC 14467	Type strain
M49 <i>M. platypocillus</i>	LA 1258		
M60 <i>M. phlei</i>	I. Baess F 89		
M98 <i>M. phlei</i>	ATCC 11758		
M83 <i>M. piscium</i>	M. Tsukamura 08006		
M50 <i>M. ranae</i>	LA 1260		
M51 <i>M. ranae</i>	LA 17		
M99 <i>M. rhodochrous</i>	ATCC 13808	R. E. Gordon, 372	
N424 <i>M. rhodochrous</i>	J. Norris ^e		
M7 <i>M. runyonii</i>	NCTC 10269	ATCC 14472	Type strain
M100 <i>M. runyonii</i>	ATCC 14472		Type strain
M32 <i>M. salmoniphilum</i>	LA 1263	ATCC 13756	Type strain
M21 <i>M. smegmatis</i>	NCTC 334		
M57 <i>M. smegmatis</i>	I. Baess F 21	ATCC 607	
M59 <i>M. smegmatis</i>	I. Baess F 87		
M63 <i>M. smegmatis</i>	I. Baess 108		
M101 <i>M. smegmatis</i>	ATCC 14468		Suggested neotype
M40 <i>M. thamnopheos</i>	LA 62		
M84 <i>M. thamnopheos</i>	M. Tsukamura 20001		
M11 <i>M. thermoresistibile</i>	NCTC 10409	ATCC 19527	Type strain
M75 <i>M. thermoresistibile</i>	M. Tsukamura 1002	ATCC 19528	
M76 <i>M. thermoresistibile</i>	M. Tsukamura 1028	ATCC 19529	
M36 <i>M. vaccae</i>	LA 2303		

TABLE 1.—Continued

Strain	Laboratory no. ^a	Source	Comment
M37 <i>M. vaccae</i>	LA 2304; R. Bönicke SN 920	ATCC 15483	Type strain
M56 <i>M. vaccae</i>	I. Baess 663; R. Bönicke SN 920	ATCC 15483	Type strain
M161 <i>M. vaccae</i>	R. Bönicke SN 920	ATCC 15483	Type strain
M162 <i>M. vaccae</i>	R. Bönicke SN 911		
M163 <i>M. vaccae</i>	R. Bönicke SN 962		
M102 <i>M. sp.</i>	ATCC 607		
M106 <i>M. sp.</i>	ATCC 23001		
M110 <i>M. sp.</i>	ATCC 23005		
M111 <i>M. sp.</i>	ATCC 23006		
M112 <i>M. sp.</i>	ATCC 23007		
M113 <i>M. sp.</i>	ATCC 23008		
M114 <i>M. sp.</i>	ATCC 23009		
N324 <i>Nocardia pellegrino</i>	ATCC 15998		
N325 <i>N. pellegrino</i>	G. Castelnouva, ^f strain 906 B		
N326 <i>N. pellegrino</i>	G. Castelnouvo 107		
N327 <i>N. pellegrino</i>	G. Castelnouva 906		
N420 <i>N. pellegrino</i>	A. Tacquet ^g strain 906		
N421 <i>N. pellegrino</i>	A. Tacquet 906 B		
N422 <i>N. pellegrino</i>	A. Tacquet 107		
N423 <i>N. pellegrino</i>	A. Tacquet 330		
N361 <i>N. rubra</i>	M. Tsukamura M 1		
N362 <i>N. rubra</i>	M. Tsukamura M 75		
N363 <i>N. rubra</i>	M. Tsukamura M 192		

^a ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; LA, Institut d'Hygiene, Lausanne, Switzerland; NCTC, National Collection of Type Cultures, London, England.

^b R. Bönicke, Institut für experimentelle Biologie und Medizin, Borstel, Germany.

^c M. Tsukamura, National Sanatorium, Chubu Chest Hospital, Obu, Aichi-ken 474, Japan.

^d I. Baess, Statens Seruminstitut, Copenhagen, Denmark.

^e "Shell" Research, Ltd., Sittingbourne, England.

^f Istituto Superiore di Sanita, Roma.

^g Institut Pasteur, Lille.

coefficients were calculated by counting positive similarities only (18) as well as by scoring positive and negative matches (21), thereby making it possible to detect cluster formation due to negative matches.

Although the main purpose of this work was classification, the taxa formed were examined, and characters were extracted from the data to facilitate the identification of the taxa.

MATERIALS AND METHODS

Strains. Sixty-nine strains of mycobacteria and ten strains of nocardiae were examined. The mycobacteria were received bearing 24 specific names and included, where possible, type or neotype strains. The name, source, and laboratory number of each organism are listed in Table 1.

Previously data had been collected on 50 "*M.*" *rhodochrous* strains and marker cultures representing the genera *Arthrobacter*, *Cellulomonas*, and *Corynebacterium*. Details about these strains, including the

results of the previous classification, are given in Table 2.

Maintenance of strains. The cultures were maintained at room temperature on two media: (i) modified Sauton's agar (14) and (ii) yeast extract agar (6).

Collection of data. All strains were examined for purity, gram-stained, and then tested for the 170 unit characters (Table 3). The resultant data were pooled with those previously collected on the "*M.*" *rhodochrous* and marker strains.

Details of the media and methods used have been described previously (6). Test media were inoculated from cultures grown for 7 days on yeast extract-agar. The tests were incubated at 37°C and read after 7 and 14 days. The nutritional and inhibition studies, and some of the biochemical tests, were carried out in divided polystyrene Replidishes. These dishes, containing 25 separate compartments, were inoculated with a simple multipoint inoculator (20). The use of this technique allows data to be collected quickly and also results in a 50% saving in the unit cost per test. The hydrolysis tests involved the measurement of zones of clearing and were carried out in petri dishes. Tests were repeated only when the results were inconclusive.

TABLE 2. Representatives of the "*Mycobacterium*" *rhodochrous* cluster, and marker strains, included in the computer analyses^a

Subgroup 14 B	
N30	" <i>M.</i> " <i>rhodochrous</i> ; R. E. Gordon, Rutgers University, New Brunswick, N.J., USA, strain A 12974. ATCC 12974.
N31	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon W 21
Subgroup 14 C	
N27	" <i>M.</i> " <i>rhodochrous</i> CCM 278
N54	" <i>M.</i> " <i>rhodochrous</i> ATCC 13808
N55	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon 817
N61	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon W 3408. ATCC 4273
N73	" <i>M.</i> " <i>rhodochrous</i> ; S. T. Williams, University of Liverpool, strain E 40
N75	" <i>M.</i> " <i>rhodochrous</i> LA 1609
N83	" <i>M.</i> " <i>rhodochrous</i> strain S. T. Williams E 40
N111	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon 562
N112	" <i>M.</i> " <i>rhodochrous</i> ; V. B. D. Skerman, University of Queensland, strain 121
N128	" <i>M.</i> " <i>rhodochrous</i> CBS 196.57
N240	" <i>M.</i> " <i>rhodochrous</i> NCTC 8571
N239	" <i>M.</i> " <i>rhodochrous</i> NCTC 8727
Subgroup 14 D	
N4	" <i>M.</i> " <i>rhodochrous</i> NCIB 9664
N11	" <i>M.</i> " <i>rhodochrous</i> NCIB 9158. ATCC 4277
N12	" <i>M.</i> " <i>rhodochrous</i> NCIB 9159
N25	" <i>M.</i> " <i>rhodochrous</i> CCM 279
N57	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon 1293S
N58	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon 1257
N59	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon 1240
N60	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon 1293 R
N63	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon 463
N64	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon 417
N65	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon A 7698. ATCC 7698
N84	" <i>M.</i> " <i>rhodochrous</i> strain S. T. Williams E 41
N108	" <i>M.</i> " <i>rhodochrous</i> strain R. E. Gordon A 4277. ATCC 4277
N143	" <i>M.</i> " <i>rhodochrous</i> ; M. Turner, University of Nottingham, England, strain 29
Subgroup 14 E	
N7	" <i>M.</i> " <i>rhodochrous</i> NCIB 10027
N10	" <i>M.</i> " <i>rhodochrous</i> NCIB 9557. ATCC 12674
N66	" <i>M.</i> " <i>rhodochrous</i> NCTC 8139
N125	" <i>M.</i> " <i>rhodochrous</i> CBS 331.61
N135	" <i>M.</i> " <i>rhodochrous</i> ; J. Antheunisse, Landbouwhogeschool, Wageningen, Holland, strain AC 338
N146	" <i>M.</i> " <i>rhodochrous</i> strain M. Turner 39
Subgroup 14 F	
N147	<i>Corynebacterium</i> sp. strain M. Turner 65
N154	<i>Arthrobacter</i> sp. strain M. Turner X 55
N155	<i>A. sp.</i> strain M. Turner X 56
Subgroup 14 G	
N170	<i>A. sp.</i> ; J. Went, ITBON, 11 Kemperbergerweg, Arnhem, Holland, strain 180
N176	<i>A. sp.</i> strain J Went 1845
N253	<i>Nocardia sp.</i> , T. Cross, University of Bradford, England, strain CUB 193
N254	<i>N. sp.</i> strain T. Cross 194
Subgroup 14 H	
N138	<i>N. sp.</i> strain J. Antheunisse R 481
N139	<i>N. sp.</i> strain J. Antheunisse R 494
Marker strains	
N291	<i>Arthrobacter globiformis</i> NCIB 8602. ATCC 4336
N295	<i>A. simplex</i> NCIB 8929. ATCC 6946
N297	<i>A. tumescens</i> NCIB 8914. ATCC 6947
N296	<i>A. ureafaciens</i> NCIB 7811. ATCC 7562 Type strain
N293	<i>Cellulomonas biazotea</i> NCIB 8077. ATCC 486
N294	<i>Mycobacterium fortuitum</i> ATCC 6841
N41	<i>Nocardia calcarea</i> NCIB 8863. ATCC 19369

^a CBS, Centraalbureau voor Schimmelcultures, Baarn, Netherlands; CCM, Czechoslovak Collection of Micro-organisms, J. E. Purkyne University, Brno; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; see also footnote a of Table 1.

Coding of characters. Most of the characters existed in one of two mutually exclusive states and were readily scored plus (+) or minus (-). Multistate characters were coded in one of two ways. Qualitative multistate characters, such as pigmentation and colony elevation, were scored plus (+) for the character state shown and minus (-) for the alternatives. Quantitative multistate characters, for example tolerance to pH and degree of acid-fastness, were coded by the additive method of Sokal and Sneath (22). The results of all the tests were entered onto standard IBM punched cards.

Computer analyses. Similarity coefficients were calculated by using the method of Sokal and Michener (21), which counts negative matches, and secondly by the method which scores only positive similarities (18). 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_d the number of nonmatching characters, then using Sokal and Michener's method, $S = (N_{sp} + N_{sn}) / (N_{sp} + N_{sn} + N_d)$, and, according to Sneath, $S = N_{sp} / (N_{sp} + N_d)$.

Sorted similarity matrices were obtained by using the unweighted average linkage cluster analysis technique. The computation was carried out on an ICL 4130 computer at Leicester University.

RESULTS

Examination of matching data, including negative matches. In the matching coefficient analysis, 122 of the 129 organisms were grouped into one of four clusters defined at the 80% level (Fig. 1). One of the large clusters (cluster 2) corresponds to the fast-growing mycobacteria, and the second (cluster 14) to "*M.*" *rhodochrous*. These two clusters join at the 67% S level. A third well-defined cluster (cluster 1) consists mainly of *Nocardia pellegrino* strains, but it also includes strains of *Nocardia rubra* and "*M.*" *rhodochrous*. Cluster 3 was ill-defined and contains strains received as arthrobacters, corynebacteria, and nocardiae.

The two larger clusters can readily be divided into subgroups at or above the 85% level. There was good overall agreement in the composition of the "*M.*" *rhodochrous* subgroups between this analysis and the previous one (6). This was not unexpected because the battery of unit characters used was very similar. The only difference was that the enlarged *N. pellegrino* taxon now formed a distinct cluster which coalesced with cluster 14 at the 68% level.

Cluster 2 consists of four subgroups. The largest, 2A, is heterogeneous and contains 21 strains carrying nine specific names. At the 90% level, strains of *M. abscessus*, *M. peregrinum*, *M. piscium*, and *M. runyonii* unite to form a tight

nucleus to the group. The other named strains included in the subgroup are *M. borstelense*, *M. chitae*, *M. gallinarum*, *M. thamnopheos*, and *M. thermoresistibile*.

Subgroup 2B is the most homogeneous and contains 24 strains. *Mycobacterium aurum*, *M. parafortuitum*, and *M. vaccae* cluster above the 90% level. *Mycobacterium diernhoferi* and two strains received as *Mycobacterium* sp. join this nucleus at a similarity of 87%.

Subgroup 2C is also homogeneous but consists of only six strains. Three of these were received as *M. fortuitum*, the others as *M. minetti*, *M. platypoecilus*, and *M. salmoniphilum*.

The final subgroup, 2D, contains ten strains. Four were received as *M. smegmatis*; the remaining named strains are *M. butyricum*, *M. lacticola*, *M. phlei*, and *M. ranae*.

There were seven unplaced organisms. These included three marker strains named *Arthrobacter globiformis*, *A. ureafaciens*, and *Cellulomonas biazotea*, and strains of *M. thamnopheos* and *M. thermoresistibile*.

Examination of the data counting positive matches only. The sorted similarity matrix (Fig. 2) was compiled from data counting positive matches only. By comparing this similarity matrix with the previous one, it can be seen that the four clusters are recovered. However, when only positive similarities are counted, the clusters are defined at a lower level of similarity.

The only significant difference between the results of the two analyses is in the composition of subgroup 2A. In the second analysis, strains of *M. borstelense*, *M. chitae*, and *M. gallinarum* are found in separate subgroups, leaving subgroup 2A as a small, homogeneous group which shares a high phenetic similarity with subgroup 2C. The similarity between subgroups 2C and 2B is equally high. In the second analysis, a strain of *M. runyonii* and one of *M. peregrinum* are found in subgroup 2C. These results suggest that, in the first analysis, some of the mycobacteria, particularly the slower growing strains, were grouped into subgroup 2A by virtue of high negative matching. The dendrograms derived by counting positive matches were then reproduced (Fig. 3 and 4). Only the clusters and subgroups defined in this way are considered further.

Identification of clusters. Certain features are common to almost all of the strains. The organisms are aerobic, gram-positive, nonmotile, oxidase negative, and grow on yeast extract-agar at pH 6 to 10 and at temperatures between 25 and 35 °C. They hydrolyze Tween

TABLE 3. *Unit characters*

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

^a Multistate quantitative character.

TABLE 3. *Continued*

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

FIG. 1. Matching coefficient analysis.

FIG. 2. Sorted similarity matrix.

20, 40, and 60 but not cellulose, chitin, gelatin, keratin, or xylan. They grow in the presence of 3% (w/v) NaCl, 0.00001% (w/v) crystal violet, 0.01% (w/v) potassium tellurite, 0.001% (w/v) sodium azide, and 0.001% (v/v) teepol but are unable to use D-arabinose, dulcitol, betaine, L-tryptophan, or serine as a sole source of carbon and energy. These tests had little discriminatory value and are omitted from further consideration.

The percentage frequency with each character was positive for the clusters shown in

Table 4. Although three of the clusters are homogeneous, few characters were found to be cluster specific. There is, however, sufficient consistency within clusters and differences between them for some characters to be weighted for identification. These characters were extracted from the data and used to construct a diagnostic table (Table 5). By using this table, strains of fast-growing mycobacteria can readily be separated from "*M.*" *rhodochrous* strains.

Identification of subgroups. In the previous

FIG. 3. Dendrogram derived by counting positive matches.

numerical analysis (6) the "*M.*" *rhodochrous* subgroups 14 A to 14 E were found to be sufficiently homogeneous for characters to be abstracted from the data and weighted for identification. Details on the properties of the subgroups and a diagnostic table for their differentiation can be found in the original paper. The properties of the subgroups of mycobacteria are shown in Table 6. Characters considered to have a presumptive diagnostic value were extracted from this table and weighted for identification (Table 7).

DISCUSSION

The numerical taxonomic evidence indicates that "*M.*" *rhodochrous* strains form a recognizable taxon clearly separated from the genus

Mycobacterium as well as from *Nocardia* (6). Although similar findings were reported by Tsukamura (27), his inclusion of "*M.*" *rhodochrous* in the newly proposed genus *Gordona* cannot be supported at present. Only six strains of "*M.*" *rhodochrous* were examined by Tsukamura, and these are too few to reflect the range of variation of a taxon which is known to be heterogeneous (6, 7). On the other hand, data from pyrolysis gas chromatography led Lechevalier et al. (13) to propose that "*M.*" *rhodochrous* be transferred to the genus *Nocardia*. Other lipid studies (14) show that nocardiae and "*M.*" *rhodochrous* strains contain a lipid which appears to be specific for nocardiae. In some of the "*M.*" *rhodochrous* strains, however, this characteristic lipid has a significantly lower R_F value than that from the *Nocardia asteroides* reference strain. At the present time, therefore, there is insufficient

FIG. 4. Dendrogram derived by counting positive matches.

evidence for placing "*M.*" *rhodochrous* in the genus *Nocardia* or in a new genus. A future generic assignment for this taxon should take into consideration the results of a subgroup which is working on these strains under the

auspices of the International Working Group on Mycobacterial Taxonomy (IWGMT).

To date, there is no objective way of equating clusters or subgroups with orthodox taxonomic rank. Even so, it would appear from

TABLE 4. *Percentage frequency of positive characters found in the four clusters*

Determination	Cluster 14, "M." <i>rhodochrous</i>	Cluster 1, <i>N. pellegri</i>	Cluster 2, <i>Mycobacterium</i> Group IV	Cluster 3, <i>Coryneforms</i>
	No. of strains tested			
	32	16	64	10
1. Mycelium, formed	100	81	14	20
2. Mycelium, temporary	100	100	100	100
3. Rods and cocci, found	100	100	83	90
4. Rods only	0	0	15	10
5. Acid-fast, weak	30	19	100	30
6. Acid-fast, strong	0	0	100	10
7. Aerial hyphae, sparse	42	50	0	20
8. Aerial hyphae, moderate	3	6	0	0
9. Colonies: White, colorless	37	0	93	90
10. Pink, orange, red	62	100	2	10
11. Yellow	0	0	3	0
12. Exopigment, water soluble	19	0	0	0
13. Colony margin: Entire	90	31	80	100
14. Filamentous	13	68	17	0
15. Deeply filamentous	0	0	2	0
16. Colonies easy to emulsify	100	100	83	100
17. Suspension, coarse	3	0	2	0
18. Colony elevation: Flat/raised	19	50	14	0
19. Convex	74	25	62	70
20. Irregular	9	25	23	30
<i>Hydrolysis Tests</i>				
21. Adenine, strong	50	0	0	0
22. Adenine, weak	70	0	0	0
23. Aesculin	100	100	62	90
24. Casein, strong	3	0	2	50
25. Casein, weak	3	0	2	60
26. DNA	64	0	0	80
27. Gelatin, strong	0	0	0	50
28. Gelatin, weak	0	0	0	60
29. Guanine	3	0	0	20
30. Hippurate	3	19	23	0
31. Hypoxanthine, strong	3	0	0	0
32. Hypoxanthine, weak	3	0	0	0
33. Tween 80	22	68	89	20
34. Tyrosine, strong	61	74	0	20
35. Tyrosine, weak	74	74	0	20
36. Xanthine, strong	3	0	0	0
37. Xanthine, weak	3	0	0	0
<i>Sole Carbon Sources</i>				
38. L-Arabinose	13	0	59	40
39. D-Arabinose	0	0	0	20
40. D-Xylose	6	0	53	30
41. L-Rhamnose	10	0	38	0
42. D-Fructose	93	100	77	90
43. D-Galactose	45	0	21	30
44. D-Glucose	100	100	97	100
45. Mannose	100	100	80	80
46. Cellobiose	13	0	20	10

TABLE 4. *Continued*

Determination	Cluster 14, "M." <i>rhodochrous</i>	Cluster 1, <i>N. pellegrino</i>	Cluster 2, <i>Mycobacterium</i> Group IV	Cluster 3, <i>Coryneforms</i>
	No. of strains tested			
	32	16	64	10
47. Lactose	6	0	2	30
48. Sucrose	84	56	34	30
49. Maltose	61	56	56	60
50. Trehalose	97	68	41	40
51. Melezitose	0	43	0	20
52. Raffinose	6	25	0	40
53. Glycogen	3	0	8	10
54. Inulin	93	100	42	0
55. Amygdalin	0	19	0	20
56. Arbutin	3	0	15	30
57. Salicin	42	0	0	0
58. Adonitol	0	25	44	0
59. Glycerol	96	94	72	80
60. Mannitol	100	100	75	70
61. Sorbitol	97	100	35	20
62. <i>meso</i> -Inositol	32	6	56	0
63. Dextrin	48	12	0	30
64. Starch	64	37	0	20
65. Glucosamine	58	0	0	50
66. Sodium acetate	93	100	80	70
67. Sodium propionate	100	100	85	70
68. Sodium- <i>n</i> -butyrate	100	100	80	70
69. Sodium- <i>n</i> -octoate	67	62	18	20
70. Sodium benzoate	45	94	44	0
71. Sodium succinate	86	100	3	10
72. Adipic acid	93	56	6	10
73. Pimelic acid	67	0	0	30
74. Sebacic acid	93	0	42	0
75. Sodium lactate	96	68	36	0
76. Sodium-H-malate	100	100	60	50
77. Sodium-D-tartrate	10	25	0	30
78. Sodium citrate	96	88	32	80
79. Sodium hippurate	3	6	9	0
80. D- α -Alanine	42	37	17	20
81. L- α -Alanine	70	0	15	0
82. DL-Nor-leucine	67	62	35	20
83. L-Threonine	16	0	0	10
84. L-Serine	42	0	11	10
85. L-Proline	42	6	35	0
86. L-Tyrosine	52	94	0	20
87. <i>p</i> -Hydroxybenzoic acid	80	81	0	0
88. <i>m</i> -Hydroxybenzoic acid	54	68	0	0
89. Protocatechuic acid	91	74	21	10
90. Testosterone	80	81	32	0
91. Sodium pyruvate	100	100	24	50
92. Acetamide	91	74	41	50
93. Benzamide	19	6	23	0
94. <i>p</i> -Cresol	54	0	0	0
95. Paraffin	100	62	0	80
96. Sodium gluconate	64	0	0	20
97. <i>o</i> -Hydroxybenzaldehyde	42	12	2	0

TABLE 4. *Continued*

Determination	Cluster 14, "M." rhodochrous	Cluster 1, N. pellegrino	Cluster 2, Mycobacterium Group IV	Cluster 3, Coryneforms
	No. of strains tested			
	32	16	64	10
<i>Sole Carbon and Nitrogen Source</i>				
98. Trimethylenediamine	0	0	2	0
99. Serine	0	0	2	0
100. Acetamide	61	31	11	0
<i>Inhibition Tests (growth on)</i>				
101. YEA containing: 5% (w/v) NaCl	100	62	47	100
102. 7% (w/v) NaCl	80	19	14	60
103. 10% (w/v) NaCl	16	0	3	20
104. 0.01% (w/v) sodium azide	77	12	15	40
105. 0.02% (w/v) sodium azide	50	6	5	20
106. 0.03% (w/v) sodium azide	3	0	2	0
107. 0.05% (w/v) potassium tellurite	86	94	97	30
108. 0.1% (w/v) potassium tellurite	70	81	75	20
109. 0.1% (v/v) phenyl ethanol	100	88	97	100
110. 0.2% (v/v) phenyl ethanol	100	50	54	90
111. 0.3% (v/v) phenyl ethanol	80	12	8	50
112. 0.4% (v/v) phenyl ethanol	6	12	2	20
113. 0.0001% (w/v) crystal violet	50	94	100	40
114. 0.001% (w/v) crystal violet	26	88	78	0
115. 0.005% (v/v) teepol	100	100	96	80
116. 0.01% (v/v) teepol	100	62	85	20
117. 0.001% (w/v) phenol	100	100	97	100
118. 0.01% (w/v) phenol	100	100	93	60
119. 0.1% (w/v) phenol	64	62	9	20
120. 0.0001% (w/v) bismuth citrate	100	100	96	100
121. 0.001% (w/v) bismuth citrate	100	74	87	40
122. 0.01% (w/v) bismuth citrate	77	19	26	40
123. 0.0001% (w/v) thallos acetate	100	100	98	100
124. 0.001% (w/v) thallos acetate	19	62	65	50
125. 0.01% (w/v) thallos acetate	0	25	30	0
126. 0.01% (w/v) tetrazolium	100	31	54	100
127. 0.1% (w/v) tetrazolium	0	0	15	0
128. Susceptibility to penicillin	100	92	0	90
129. Growth at: 50 C	3	0	0	0
130. 45 C	10	25	6	0
131. 40 C	100	88	44	40
132. 10 C	96	25	2	80
133. Benzidine, strong	100	88	33	50
134. Benzidine, weak	100	100	90	100
135. Oxidase	0	0	0	10
136. Catalase, strong	100	100	86	30
137. Nitrate reduction	100	62	75	80
138. Urease, strong	48	12	63	10
139. Urease, weak	54	12	66	20
140. Allantoinase, strong	3	0	0	0
141. Allantoinase, weak	42	0	0	0
142. Lysozyme resistant	0	0	14	10
143. <i>p</i> -Nitrophenoloxidase	10	0	2	0
144. <i>m</i> -Nitrophenoloxidase	19	50	2	0

TABLE 5. *Characteristics of the clusters*

Determination	Cluster 14, "M." <i>rhodochrous</i>	Cluster 1, <i>N. pellegrino</i>	Cluster 2, <i>Mycobacterium</i> Group IV.	Cluster 3, coryneforms
	No. of strains tested			
	32	16	64	10
1. Mycelium, formed .	+ ^a	+	-	-
6. Acid-fast, strong ..	-	-	+	-
9. Colonies—white, colorless	v	-	+	+
<i>Sole Carbon Sources</i>				
54. Inulin	+	+	v	-
71. Sodium succinate ..	+	+	-	-
72. Adipic acid	+	v	-	-
74. Sebacic acid	+	-	-	-
86. L-Tyrosine	v	+	-	v
95. Paraffin	+	v	-	+
128. Susceptibility to penicillin	+	+	-	+
132. Growth at 10 C	+	v	-	+

^a Plus (+) or minus (-) means that 85% or more of the strains give that result; v, variable, i.e., 16 to 84% of strains give a positive result.

the results of this work that too many species have been suggested for fast-growing mycobacteria. Some of the subgroups are homogeneous and can be compared to species even though they contain strains which belong to two or more named species. Subgroups based upon overall similarity are more reliable than taxa defined on a few properties, and they accommodate a degree of individual strain variability. It is doubtful, therefore, whether the differences between some of the taxa of mycobacteria are sufficient to consider the taxa as species.

Subgroup 2B is homogeneous and can probably be equated with a species. In this case *M. aurum*, *M. diernhoferi* and *M. parafortuitum* can be reduced to synonyms of *M. vaccae*, a name which has priority over the others. Additional evidence drawn from numerical taxonomy lends support to some of these proposals. Thus, although *M. aurum* and *M. parafortuitum* were described by Tsukamura (23, 24), he and his colleagues later included them in a single species, *M. parafortuitum* (28).

These workers also considered that the differences observed between *M. diernhoferi* and *M. parafortuitum* might be due solely to differences in the rates of growth of these organisms.

Subgroup 2C is equivalent to the established species *M. fortuitum*. This taxon includes the type strains of *M. minetti*, *M. peregrinum*, and *M. salmoniphilum*. Several workers have already considered *M. minetti* as belonging to *M. fortuitum* (4, 8, 10, 12, 28), and Ross (16) was able to distinguish *M. salmoniphilum* from this species only by two characters.

Similarly, subgroup 2D can be considered equivalent to *M. smegmatis*, but this subgroup also contains strains named *M. butyricum*, *M. lacticola*, and strains mislabeled as *M. ranee*. Strains of these species have frequently been recognized as strains of *M. smegmatis* (4, 10, 28). When positive matches only are counted, subgroup 2A is recovered as a tight group containing strains named *M. abscessus*, *M. piscium*, and *M. runyonii*. Some of these strains share a high overall similarity with strains of *M. fortuitum*, from which they are difficult to distinguish. This result leaves the taxonomic state of *M. abscessus* (*M. runyonii*) confused, for, while Tsukamura (28, 29) reduced the name *M. abscessus* to a synonym of *M. fortuitum*, Nagayama et al. (15) regarded *M. abscessus* as a separate species. In the second analysis, *M. borstelense*, *M. chitae*, and *M. gallinarum* fell into subgroups which were separate both from one another and from subgroup 2A. This result supports the finding that *M. abscessus* and *M. borstelense* are distinct species (26), though data from lipid and serological tests have led to the recommendation that *M. borstelense* be recognized as a subspecies of *M. abscessus* (11). The strains in these subgroups are relatively slow growers, and cluster formation may have been influenced by this factor. Although these subgroups probably represent distinct species, the degree of variation between them should be separated into the two components of vigor and pattern (19) to ensure that this is so.

It was possible to weight characters for the identification of clusters and for the subgroups of mycobacteria. In particular, strains of "M." *rhodochrous* can now be readily separated from fast-growing mycobacteria. In addition to classical tests, such as the degree of acid-fastness, some of the newly applied characters were found to have a differential value. Some of the carbon compounds used as sole sources of carbon and energy are prominent in the diagnostic tables. It was, however, found that

TABLE 6. *Percentage frequency of positive characters in each subgroup of Mycobacterium*

Determination	Subgroup							
	2A	2B	2C	2D	2E	2F	2G	2H
	No. of strains tested							
	7	23	8	12	4	2	2	4
1. Mycelium, formed	0	0	62	25	0	0	0	0
2. Mycelium, temporary	0	0	100	100	0	0	0	0
3. Rods and cocci, formed	56	100	75	83	100	100	100	100
4. Rods, only	43	0	25	17	0	0	0	0
9. Colonies: White, colorless	100	82	100	92	100	100	0	100
10. Orange, pink, red	0	0	0	8	0	0	100	0
11. Yellow	0	10	0	0	0	0	0	0
13. Colony margin: Entire	84	100	62	50	50	100	0	100
14. Filamentous	0	0	38	50	50	0	100	0
15. Deeply filamentous	14	0	0	0	0	0	0	0
16. Colonies easy to emulsify	100	91	0	100	100	100	100	100
17. Suspension, coarse	14	0	0	0	0	0	0	0
18. Colony elevation: Flat/raised	0	13	12	50	0	0	0	0
19. Convex	84	84	25	8	100	50	50	100
20. Irregular	14	4	62	42	0	50	50	0
<i>Hydrolysis Tests</i>								
23. Aesculin	100	53	88	58	100	100	0	100
24. Casein, weak	0	0	0	0	25	0	0	0
25. Casein, strong	0	0	0	0	25	0	0	0
30. Hippurate	28	40	0	8	0	100	0	0
33. Tween, 80	56	100	88	92	100	100	100	100
<i>Sole Carbon Source</i>								
38. L-Arabinose	0	88	12	92	0	100	100	0
40. D-Xylose	14	88	0	92	0	0	50	0
41. L-Rhamnose	42	65	0	100	0	0	100	0
42. D-Fructose	0	100	100	100	100	50	0	0
43. D-Galactose	0	0	12	92	0	0	0	0
45. D-Mannose	84	93	100	100	0	50	50	100
46. Cellobiose	0	22	12	42	0	0	0	0
47. Lactose	0	0	0	8	0	0	0	0
48. Sucrose	0	69	0	50	0	0	0	0
49. Maltose	0	65	75	92	25	50	0	25
50. Trehalose	0	65	25	75	0	0	50	0
53. Glycogen	0	0	0	34	0	0	0	0
54. Inulin	0	61	25	75	50	0	0	0
56. Arbutin	28	0	38	25	0	0	0	0
58. Adonitol	0	88	0	92	25	0	0	0
59. Glycerol	70	88	100	100	0	100	0	0
60. Mannitol	14	91	62	92	100	100	100	0
61. Sorbitol	28	26	0	92	0	50	0	0
62. <i>meso</i> -Inositol	0	88	12	92	100	100	50	0
66. Sodium acetate	84	88	100	92	100	100	0	0
67. Sodium propionate	70	96	100	100	100	0	50	0
68. Sodium- <i>n</i> -butyrate	42	84	100	100	50	50	100	50
69. Sodium- <i>n</i> -octoate	14	4	50	58	0	0	50	0
70. Sodium benzoate	14	53	0	92	100	0	100	0
71. Sodium succinate	0	0	12	0	0	0	0	0
72. Adipic acid	0	0	50	0	0	0	0	0
74. Sebacic acid	28	22	88	100	0	0	0	0
75. Sodium lactate	14	35	38	66	100	0	0	0
76. Sodium-H-malate	0	78	88	92	0	0	0	0
78. Sodium citrate	0	50	62	66	0	0	0	25
79. Sodium hippurate	0	0	0	17	0	0	0	0
80. D- α -Alanine	0	0	25	58	0	0	0	0
81. DL-Nor-leucine	14	31	88	42	0	0	0	0
82. L- α -Alanine	0	13	25	42	0	0	0	0

TABLE 6. *Continued*

Determination	Subgroup							
	2A	2B	2C	2D	2E	2F	2G	2H
	No. of strains tested							
	7	23	8	12	4	2	2	4
84. L-Serine	0	9	12	32	0	0	0	0
85. L-Proline	0	18	75	92	0	0	0	0
89. Protocatechuic acid	14	31	0	58	0	0	0	0
90. Testosterone	28	40	88	8	0	0	0	0
91. Sodium pyruvate	0	26	0	75	0	0	0	0
92. Acetamide	0	22	75	100	50	0	0	0
93. Benzamide	14	22	0	62	0	0	50	0
97. <i>o</i> -Hydroxybenzaldehyde	0	0	0	8	0	0	0	0
<i>Sole Carbon and Nitrogen Source</i>								
99. Trimethylenediamine	7	0	0	0	0	0	0	0
99. Serine	0	0	0	8	0	0	0	0
100. Acetamide	0	0	50	8	50	0	0	0
<i>Inhibition Tests (growth on)</i>								
101. YEA containing: 5% (w/v) NaCl	56	26	62	100	75	50	0	25
102. 7% (w/v) NaCl	14	9	12	42	25	0	0	0
103. 10% (w/v) NaCl	0	4	0	0	0	0	0	0
104. 0.01% (w/v) Sodium azide	28	9	25	32	0	50	100	0
105. 0.02% (w/v) Sodium azide	0	4	25	0	0	0	0	0
106. 0.03% (w/v) Sodium azide	0	0	12	0	0	0	0	0
107. 0.05% (w/v) Potassium tellurite	84	100	100	100	100	100	100	100
108. 0.1% (w/v) Potassium tellurite	56	100	100	83	100	50	100	100
109. 0.1% (v/v) Phenyl ethanol	100	100	100	100	50	100	100	100
110. 0.2% (v/v) Phenyl ethanol	42	53	62	100	0	50	0	50
111. 0.3% (v/v) Phenyl ethanol	0	4	0	42	0	0	0	0
112. 0.4% (v/v) Phenyl ethanol	0	0	0	8	0	0	0	0
114. 0.001% (w/v) Crystal violet	100	85	100	92	100	50	0	50
115. 0.005% (v/v) Teepol	100	91	100	100	100	100	100	100
116. 0.01% (v/v) Teepol	70	85	88	100	0	100	100	100
117. 0.001% (v/v) Phenol	100	100	100	100	100	100	100	100
118. 0.01% (w/v) Phenol	100	100	100	100	100	50	25	
119. 0.1% (w/v) Phenol	0	0	0	58	0	0	0	0
120. 0.0001% (w/v) Bismuth citrate	100	100	100	100	100	100	100	25
121. 0.001% (w/v) Bismuth citrate	100	96	100	100	100	100	100	0
122. 0.01% (w/v) Bismuth citrate	56	0	50	58	0	100	0	0
123. 0.0001% (w/v) Thallous acetate	100	100	100	100	100	100	100	75
124. 0.001% (w/v) Thallous acetate	70	100	100	83	50	100	0	25
125. 0.01% (w/v) Thallous acetate	56	44	88	17	0	100	0	0
126. 0.01% (w/v) Tetrazolium	100	44	75	92	25	50	0	0
127. 0.1% (w/v) Tetrazolium	53	0	12	50	0	0	0	0
<i>Growth at (temp)</i>								
130. 45 C	0	0	0	50	0	0	0	0
131. 40 C	56	31	50	92	100	0	0	0
132. 10 C	14	0	0	0	0	0	0	0
<i>Biochemical Tests</i>								
133. Benzidine, strong	0	17	62	100	0	0	50	0
134. Benzidine, weak	100	91	100	100	100	0	100	100
136. Catalase, strong	100	84	88	92	100	100	50	100
137. Nitrate reduction	70	70	100	83	50	100	50	100
138. Urease, strong	56	91	75	100	0	100	0	25
139. Urease, weak	56	91	75	100	0	100	50	25
142. Lysozyme resistant	56	0	50	0	0	0	0	0
143. <i>p</i> -Nitrophenoloxidase	0	0	12	0	0	0	0	0
144. <i>m</i> -Nitrophenoloxidase	0	4	0	0	0	0	0	0

TABLE 7. Characteristics of the subgroups of "Mycobacterium"

Determination	Subgroup							
	2A	2B	2C	2D	2E	2F	2G	2H
	No. of strains tested							
	7	23	8	12	4	2	2	4
9. Colonies: White, colorless	+	+	+	+	+	+	—	+
10. Orange, pink, red	—	—	—	—	—	—	+	—
30. Hydrolysis of hippurate	v	v	—	—	—	+	—	—
<i>Sole Carbon Source</i>								
38. L-Arabinose	—	+	—	+	—	+	+	—
40. D-Xylose	—	+	—	+	—	—	v	—
41. L-Rhamnose	v	v	—	+	—	—	+	—
42. D-Fructose	—	+	+	+	+	v	—	—
43. D-Galactose	—	—	—	+	—	—	—	—
45. D-Mannose	+	+	+	+	—	v	v	+
58. Adonitol	—	+	—	+	v	—	—	—
59. Glycerol	v	+	+	+	—	+	—	—
60. Mannitol	—	+	v	+	+	+	+	—
62. meso-Inositol	—	+	—	+	+	+	v	—
66. Sodium acetate	+	+	+	+	+	+	—	—
67. Sodium propionate	v	+	+	+	+	—	v	—
70. Sodium benzoate	—	v	—	+	+	—	+	—
74. Sebacic acid	v	v	+	+	—	—	—	—
76. Sodium-H-malate	—	v	+	+	—	—	—	—
90. Testosterone	v	v	+	—	—	—	—	—
131. Growth at 40 C.	v	v	v	+	+	—	—	—
139. Urease, weak	v	+	v	+	—	+	v	v

^a Plus (+) or minus (—) means that 85% or more of the strains give that result; v, variable, i.e., 16 to 84% of strains give a positive result.

the results of some tests did not always correspond with those obtained by other workers (11, 15, 27, 28). At present, little work has been done to assess the reproducibility of simple phenotypic characters considered to have a diagnostic value. Now that several large recognizable groups of nocardiae and mycobacteria are emerging, the search for reliable diagnostic characters can be intensified.

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