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A Co-operative Numerical Analysis of Cultures Considered to Belong to the '*rhodochrous*' Taxon

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SUMMARY

A co-operative taxonomic study has been performed on cultures belonging to the 'rhodochrous complex'. Phenetic data on 98 cultures (78 rhodochrous cultures, 12 marker cultures with the genus designation *Mycobacterium* and 8 with the designation *Nocardia*) studied in four laboratories were collected and analysed by numerical taxonomic methods. The precipitinogenic properties and the presence of different types of mycolic acids were analysed independently to establish correlation with the numerical classification. The chemotaxonomic and serological data correlated well with the numerical analyses. The rhodochrous taxon can be distinguished from the genera *Mycobacterium* and *Nocardia*, and from the proposed genus *Actinomadura*; furthermore, it can be divided into at least three homogeneous subgroups. Further studies however, are needed before the question of the generic location of the rhodochrous taxon can be settled and before the taxonomic status of the rhodochrous subclusters can be resolved.

INTRODUCTION

The International Working Group on Mycobacterial Taxonomy (IWGMT) has carried out a number of co-operative taxonomic studies in an attempt to improve the classification and identification of species within the genus *Mycobacterium* (Wayne *et al.* 1971; Kubica *et al.* 1972; Meissner *et al.* 1974). At an early stage it was recognized that not only species which indisputably belonged to the genus *Mycobacterium* had to be considered but also taxa with no settled generic niche such as the rhodochrous taxon or 'rhodochrous complex'. The organisms in this group have been classified in the genus *Mycobacterium* (Gordon & Mihm, 1957) and in the genus *Nocardia* (Lechevalier, Horan & Lechevalier, 1971), while Gordon & Mihm (1959) characterized the taxon as a 'species in search of a genus'. By 1969 no definitive numerical taxonomic study had been reported on the rhodochrous taxon, so the IWGMT decided to organize a co-operative numerical analysis of cultures considered to belong to the 'rhodochrous complex'. This paper gives the results obtained in a study in which workers from five different laboratories (coded A to E) participated. This investigation was performed using the same 'permissive' philosophy (Wayne *et al.* 1971) adopted in the earlier studies.

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Table 1. *Species designations and sources of marker cultures*

ATCC no. of source	Designation	ATCC no. of source	Designation
6841	*Mycobacterium fortuitum*	23366	*Mycobacterium aurum*
11758	*Mycobacterium phlei*	25969	*Mycobacterium album* (from R. E. Gordon)
14468	*Mycobacterium smegmatis*	14629	*Nocardia caviae*
14474	*Mycobacterium flavescens*	14816	*Nocardia pelletieri*
15483	*Mycobacterium vaccae*	19247	*Nocardia asteroides*
19235	*Mycobacterium borstelense*	19296	*Nocardia brasiliensis*
19340	*Mycobacterium diernhoferi*	19425	*Nocardia madurae*
19527	*Mycobacterium thermoresistible*	23219	*Nocardia dassonvillei*
19686	*Mycobacterium parafortuitum*	25970	*Nocardia farcinica* (from R. E. Gordon)
23023	*Mycobacterium peregrinum*	25971	*Nocardia farcinica* (from R. E. Gordon)

METHODS

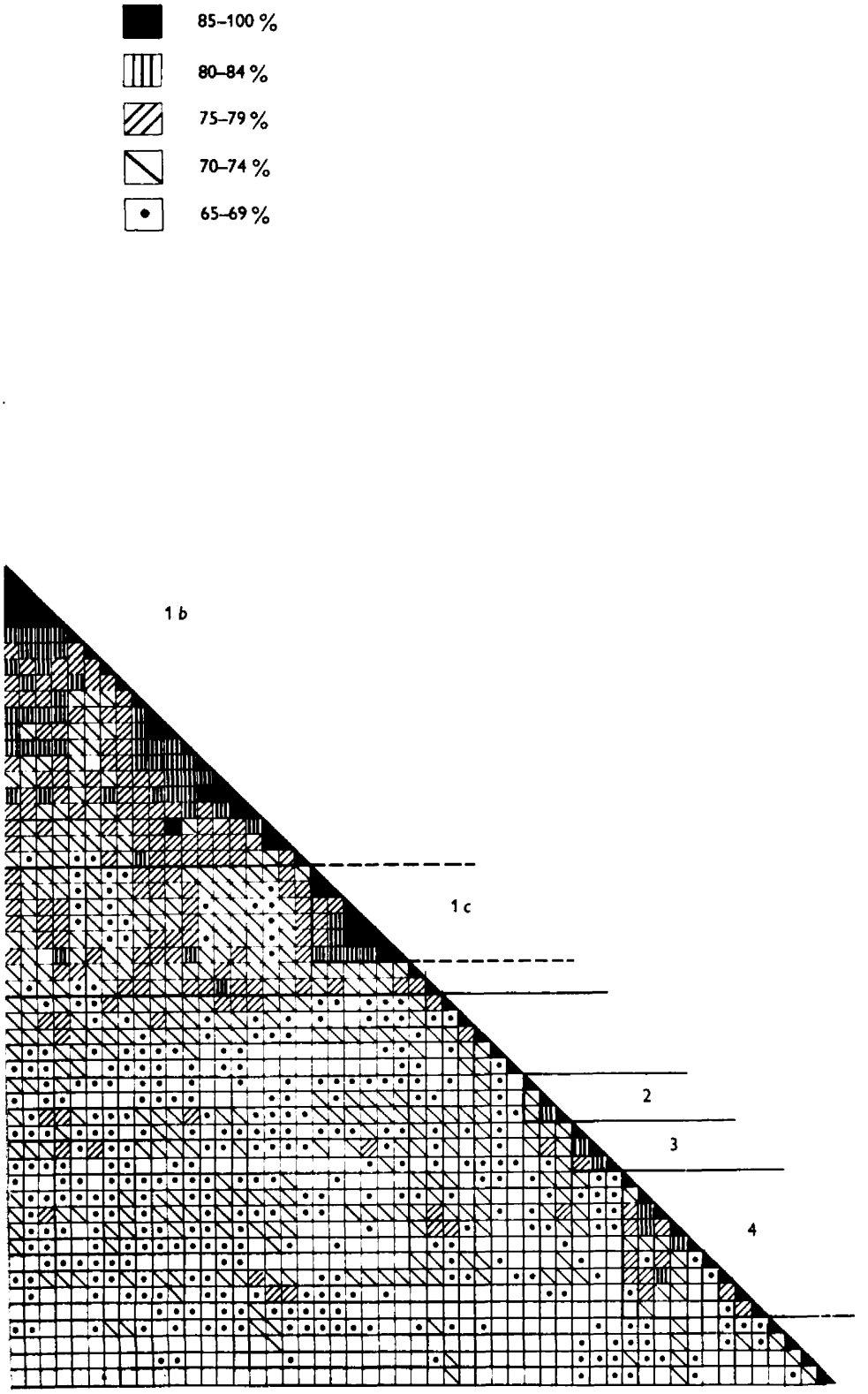
Bacterial cultures. One hundred cultures representing the 'rhodochrous complex' and allied taxa were selected from three collections, the American Type Culture Collection, ATCC (32 cultures), the U.S. Department of Health Education, and Welfare Center for Disease Control, C.D.C. (28 cultures), and the collection of Dr R. E. Gordon, R.E.G. (40 cultures). All cultures were lyophilized, coded and distributed by the ATCC as before (Wayne *et al.* 1971).

Eighty of the test cultures were assigned to the 'rhodochrous complex' (Table 2*a*, *b*, *c* below) and of the 20 marker strains 12 had been classified in the genus *Mycobacterium*; most of them were rapidly growing mycobacteria belonging to Runyon's group IV (Kubica *et al.* 1972) and eight were in the genus *Nocardia* (Table 1). Where possible the marker strains were type cultures. Out of the 80 rhodochrous cultures, 28 were designated as 'rhodochrous-like strains', two as 'unidentified strains', whereas the remaining 50 cultures carried 30 different species epithets. Six of the eighty rhodochrous cultures were included as duplicates or triplicates (Table 2*a*, *b*).

Collection and coding of data. Participants studied the cultures using tests of their own choosing. As before (Wayne *et al.* 1971), participants agreed not to withdraw their data regardless of the final result and agreed that no details of the techniques used would be presented at this stage. The individual batches of data were sent to the co-ordinators who recorded the relevant information into binary form for the numerical analyses. Four laboratories submitted a total of 272 characters per culture but this data included both 'irrelevant' and 'repetitious' characters. Tests in which all of the cultures gave the same result, that is either all positive or all negative responses, were deleted as irrelevant as they have no differential value. Fifty-six tests were carried out by more than one investigator and as they gave substantially similar results only those from a single laboratory were retained. Repetitious data must be eliminated in order to avoid undue weighting of characters. The final $n \times t$ matrix contained 180 characters and 98 operational taxonomic units, o.t.u. (i.e. cultures). The findings of the chemical and serological studies were not included in the pooled data but were reserved for comparison with the results of the numerical analyses.

Computer analyses. The data were examined using the matching coefficient, S_M, which counts both positive and negative similarities. Initially trials were also made with the similarity coefficient, S_J, which excludes negative matches, but the results obtained were very similar to those obtained with the S_M coefficient and therefore revealed no important additional points of interest. Conventional sorted similarity matrices were obtained using both

Fig. 1. Matching matrix of the 98 te



st cultures based on 180 pooled characters.

single linkage and unweighted average linkage clustering (Sokal & Sneath, 1963). Since the results of the two clustering techniques were virtually the same only those from the average linkage are considered here.

RESULTS

Numerical analysis of pooled data from laboratories A to D

Analysis of 180 unit characters and 98 o.t.u. resolved the cultures into four clusters defined at the 75 to 80 % similarity level (S-level) (Fig. 1). Most of the cultures in the largest cluster, cluster 1, fell into one of three subclusters, 1*a*, 1*b* or 1*c* (Table 2*a*,*b*,*c*). The mean intra-group similarity of each cluster and subcluster, and the corresponding inter-group similarities are shown in Table 3. With the exception of cluster 4 (at 71 %) mean intra-group similarities were high ($\geqslant$ 77 %).

Cluster 1 contains 74 of the 79 cultures provisionally assigned to the 'rhodochrous complex'. Thirty-seven of these cultures fell into subcluster 1*a* (Table 2*a*), 29 into 1*b* (Table 2*b*), 6 into 1*c* (Table 2*c*), while 2 cultures possibly formed the nucleus of a fourth subcluster (Table 2*c*). None of the marker strains were recovered in this cluster. The mean intra-group similarities for the three subgroups in cluster 1 are 81, 79 and 83 % respectively (Table 3), clearly separating this cluster from clusters 2, 3 and 4 ($\leqslant$ 70 %).

Cluster 2 contains three cultures belonging to Runyon's group IV, namely *Mycobacterium borstelense* (ATCC19235), *M. fortuitum* (ATCC6841) and *M. peregrinum* (ATCC23023).

Cluster 3 consists of three nocardiae cultures, *Nocardia asteroides* (ATCC19247), *N. brasiliensis* (ATCC19296) and *N. caviae* (ATCC14629).

Cluster 4 includes nine cultures. Eight are rapidly growing mycobacteria: *Mycobacterium aurum* (ATCC23366), *M. diernhoferi* (ATCC19340), *M. flavescens* (ATCC14474), *M. parafortuitum* (ATCC19686), *M. phlei* (ATCC11758), *M. smegmatis* (ATCC14468), *M. thermoresistibile* (ATCC19527), and *M. vaccae* (ATCC15483); the ninth is one of the two *Nocardia farcinica* cultures (ATCC25970).

Nine cultures were not recovered in the defined clusters, namely *Corynebacterium fascians* (ATCC25738), *Mycobacterium album* (ATCC25969), *Nocardia dassonvillei* (ATCC23219), *N. farcinica* (ATCC25971), *N. madurae* (ATCC19425), *N. pelletieri* (ATCC14816), *N. rhodnii* (ATCC25735), and two 'rhodochrous-like strains' (ATCC25691 and ATCC25695).

Numerical analysis of data from laboratory A. In this laboratory the cultures were examined for 104 characters, 12 of which were deleted as irrelevant. Nintey-three of the 98 cultures studied fell into five clusters defined at the 75 to 80 % S-level. The largest cluster was sharply separated from the other clusters and contained 77 cultures initially assigned to the 'rhodochrous complex'. Most of the rhodochrous cultures were recovered in three homogeneous subclusters which were almost identical in composition to those obtained from the analysis of the pooled data. A fourth but diffuse subcluster was formed by eight cultures, namely *C. fascians* (ATCC25738 and ATCC25739), *Nocardia* sp. (ATCC25707), *M. album* (ATCC25969), *Proactinomyces* spp. (ATCC25708, ATCC25709 and ATCC25710) and a 'rhodochrous-like strain' (ATCC25683). All the duplicate and triplicate cultures were found in the relevant subcluster.

Most of the marker strains were recovered in the four remaining clusters. The three Nocardia cultures were again recovered as a distinct cluster, and most of the mycobacteria were once more distributed between two clusters. In this analysis, however, a fifth cluster was formed comprising *Mycobacterium vaccae* (ATCC15483) and a 'rhodochrous-like strain' (ATCC25691). The cultures representing *Nocardia dassonvillei, N. farcinica* (ATCC25971), *N. madurae, N. pelletieri* and *N. rhodnii* once again remained outside the defined clusters.

Table 2. *Cultures assigned to cluster* 1

(*a*) Cultures assigned to subcluster 1*a*

ATCC no.	Designation and source
4001	*Bacillus havaniensis*
999*	*B. mycoides corallinus*
4004	*B. mycoides roseus* I
271	*B. mycoides roseus* II
14341	*B. rubricus*
19067	*Corynebacterium* sp.
25721	*Jensenia* sp., R.E.G., 885
25700	*Micrococcus rhodochrous*; R.E.G., W21
4273†	*Mycobacterium agreste* = 335, 338 (Bradley, 1971) = N22, N61, N84 (Goodfellow, 1971)
4276	*M. crystallophagum* = 337 (Bradley, 1971)
25717	*M. lacticola*; R.E.G., 584
25737	*M. lacticola*; R.E.G., NCTC8154
25726	*M. rhodochrous*; R.E.G., 1095
14343	*M. rubropertinctum*
25706	*Mycobacterium* sp; R.E.G., 463
14347	*Nocardia corallina*
14348	*N. corallina*
14349	*N. corallina*
14350	*N. corallina*
25712†	*N. corallina*; R.E.G., 502
25719*	*N. corallina*; R.E.G., 624
25730*	*N. corallina*; R.E.G., W3406
25732†	*N. corallina*; R.G.E., W3408
25731	*N. erythropolis*; R.E.G., W3407 = ATCC4277 = 474 (Bradley, 1971) = N23, N108 (Goodfellow, 1971)
25703	*N. rubra*; R.E.G., 415
25733	*N. rubra*; R.E.G., W3639 = N110 (Goodfellow, 1971)
25707	*Nocardia* sp; R.E.G., 482
25724	*Nocardia* sp; R.E.G., 1082R
25725	*Nocardia* sp; R.E.G., 1082S
25708	*Proactinomyces erythropolis*; R.E.G., 492
25709	*Proactinomyces* sp; R.E.G., 493
13808	*Rhodococcus rhodochrous* = N54 (Goodfellow, 1971)
25680	'*rhodochrous*'; C.D.C., T2568–6
25686	'*rhodochrous*'; C.D.C., Rhod. 2
25692	'*rhodochrous*'; C.D.C., T227–6
25696	'*rhodochrous*'; C.D.C., T2059–6
25722	Unidentified strain; R.E.G., 1022

(*b*) Cultures assigned to subcluster 1*b*

ATCC no.	Designation and source
25705	*Mycobacterium eos*; R.E.G., 462
25723‡	*M. rhodochrous*; R.E.G., 1054
25702	*M. rubrum*; R.E.G., 384
25736	*Mycobacterium* sp., R.E.G., NCTC8139
25714	*Nocardia globerula*; R.E.G., 544
25710	*Proactinomyces globerula*; R.E.G., 494 = N109 (Goodfellow, 1971)
25711	*P. globerula*; R.E.G., 495
25669	'*rhodochrous*'; C.D.C., T334–5
25670	'*rhodochrous*'; C.D.C., T454–5
25671	'*rhodochrous*'; C.D.C., T456–5
25672	'*rhodochrous*'; C.D.C., T644–5
25673	'*rhodochrous*'; C.D.C., T260–6
25674	'*rhodochrous*'; C.D.C., T262–6
25675	'*rhodochrous*'; C.D.C., T258–6
25676	'*rhodochrous*'; C.D.C., T2060–6
25678	'*rhodochrous*'; C.D.C., T2338–6
25679	'*rhodochrous*'; C.D.C., T2544–6
25681	'*rhodochrous*'; C.D.C., T2569–6
25682	'*rhodochrous*'; C.D.C., T610–7
25683	'*rhodochrous*'; C.D.C., T875–7
25684‡	'*rhodochrous*'; C.D.C., T957–7
25685	'*rhodochrous*'; C.D.C., T452–8
25687	'*rhodochrous*'; C.D.C., T312–2
25688	'*rhodochrous*'; C.D.C., T394–2
25689	'*rhodochrous*'; T572–5
25698	'*rhodochrous*'; C.D.C., T954–7
25699	'*rhodochrous*'; C.D.C., T980–7
25701	Unidentified strain; R.E.G., 382
25727	*Corynebacterium rubrum*; R.E.G., 1240 = N59 (Goodfellow, 1971)

(*c*) Cultures assigned to subcluster 1*c*, to cluster 1 and those not recovered in a defined cluster

ATCC no.	Designation and source
	Subcluster 1*c*
25694§	*Corynebacterium equi*, C.D.C., T675–6
25729	*C. equi*, R.E.G., 1621
25734§	*C. equi*, R.E.G., NCTC4219
25715	*Nocardia restrictus*; R.E.G., 545
25728	*N. restrictus*; R.E.G., 1256
25716	*Proactinomyces restrictus*; R.E.G., 566
	Cluster 1
25690	'*rhodochrous*'; C.D.C., T595–5
25739	*Corynebacterium fascians*; R.E.G., 1300
	Not clustered
25691	'*rhodochrous*'; C.D.C., T204–6
25695	'*rhodochrous*'; C.D.C., T2054–6
25735	*Nocardia rhodnii*; R.E.G., NCTC6117
25738	*Corynebacterium fascians*; R.E.G., 12974

ATCC184 *Micrococcus rhodochrous* and ATCC25704 *Nocardia globerula* (R.E.G., 417) were excluded as insufficient data was received on them.

*, † Triplicate cultures.

‡, § Duplicate cultures.

Table 3. *Similarity levels (% S-level)*

These are expressed as mean inter- and intra-group similarities, with standard deviations, for the six clusters and subclusters. The S-levels are rounded to the nearest 1 %.

Cluster 1*a*	81±4·8					
1*b*	75±4·1	79±5·4				
1*c*	74±3·9	74±3·3	83±4·1			
2	66±4·2	68±4·1	70±2·5	77±3·7		
3	67±3·3	68±3·9	68±3·7	74±2·7	80±2·3	
4	65±5·1	67±3·7	65±3·9	67±5·5	65±4·7	71±8·2
	1*a*	1*b*	1*c*	2	3	4

Numerical analysis of data from laboratory B. In this laboratory the cultures were examined for 108 characters, but for computation 20 of these were deleted because they gave results which were either 100 % positive or 100 % negative. At the 75 to 80 % S-level five clusters were defined. The rhodochrous cultures were again found in three subclusters within a cluster sharply separated from the remaining clusters. Cluster 2 was recovered as in the analysis of the pooled data but also contained two additional marker cultures, *M. parafortuitum* and *N. farcinica* (ATCC25791). The three Nocardia cultures were again found in cluster 3 but the second Mycobacterium cluster was smaller than before and only contained four cultures, *M. aurum*, *M. diernhoferi*, *M. phlei* and *M. vaccae*. The fifth cluster was heterogeneous and contained the slower-growing cultures *M. flavescens*, *M. thermoresistibile*, *N. pelletieri* and the rhodochrous culture ATCC25705. In this analysis the marker strains *N. dassonvillei*, *N. farcinica* (ATCC25970), *N. madurae* and *M. smegmatis* were not grouped in any of the defined clusters.

Numerical analysis of data from laboratory C. In this laboratory the test cultures were examined for 28 unit characters including four serological ones. Three clusters were recovered at the 75 to 80 % S-level. Once more all the rhodochrous cultures were found in a distinct cluster, but in this analysis the four marker cultures *M. abscessus*, *M. diernhoferi*, *M. flavescens* and *M. parafortuitum* were also included in cluster 1. Subclusters 1*a* and 1*b* were again distinguishable but the final subcluster 1*c* was now found as a recognizable entity in subcluster 1*a*. With a single exception, the duplicate and triplicate cultures were found in the relevant subcluster.

Cluster 3 was again recovered, but in this analysis also contained the marker strains *N. dassonvillei*, *N. madurae*, and *N. pelletieri*. With a single exception, the Mycobacterium marker cultures and *N. farcinica* (ATCC25971) were grouped together in a large heterogeneous cluster. The remaining culture, *M. thermoresistible*, did not fall into any of the defined clusters.

Numerical analysis of data from laboratory D. The data from this laboratory were used to form an $n \times t$ matrix consisting of 98 cultures and 32 unit characters. The results were very similar to those from laboratory C. The rhodochrous cultures again fell into a distinct cluster and the subclusters were again recognizable. The Nocardia and Mycobacterium marker cultures were separate from one another and from the rhodochrous cultures.

Comparative analyses of independent data

Serological studies performed by laboratory C. This laboratory also analysed the cultures serologically by means of a comparative immuno-diffusion method using 12 serological reference systems representing *Mycobacterium bovis* var. *BCG*, *M. microti*, *M. kansasii*, *M. marianum*, *M. avium*, *M. fortuitum*, *M. phlei*, *N. asteroides*, *N. caviae*, and three members

Table 4. *Number of cultures showing cross-reacting precipitinogens to mycobacterial,* rhodochrous and nocardia cultures used as references for comparative immunodiffusion analyses*

No. of precipitates of the reference system showing deviation phenomena are given in parentheses.

		Species									
		M. bovis var. *BCG*	*M. microti*	*M. marianum*	*M. avium*		*M. fortuitum*				*M. phlei*
Cluster	No. of cultures	(1)	(1)	(1)	(1)	(2)	(1)	(2)	(3)	(5)	(1)
1*a*	37	2	—	—	—	—	1	—	—	—	—
1*b*	29	3	—	—	—	—	—	—	—	—	—
1*c*	6	1	—	—	—	—	—	—	—	—	—
1†	2	—	—	1	—	—	—	—	—	—	—
2	3	1	—	—	—	3	1	—	—	1	—
3	3	—	—	1	1	1	—	—	—	—	—
4	9	3	1	2	3	1	—	3	—	—	1
Unclustered	9	1	—	1	—	1	—	—	1	—	—

		'*M.*' *rhodochrous*					'*N.*' *pellegrino*				'*N.*' *polychromogenes*				
		(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(5)
1*a*	37	24	2	1	1	—	11	5	1	1	—	—	—	—	—
1*b*	29	2	16	5	1	1	1	1	—	—	—	2	5	6	6
1*c*	6	5	—	—	—	—	1	—	—	—	—	—	—	—	—
1†	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—
2	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	3	—	—	—	—	—	2	—	—	—	—	—	—	—	—
4	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Unclustered	9	4	—	—	—	—	3	—	1	—	—	—	—	—	—

		N. asteroides			*N. caviae*	
		(1)	(2)	(3)	(1)	(6)
1*a*	37	8	1	—	—	—
1*b*	29	14	5	—	4	—
1*c*	6	—	—	—	—	—
1†	2	—	—	—	—	—
2	3	—	—	—	—	—
3	3	1	1	1	—	1
4	9	1	1	—	3	—
Unclustered	9	2	—	—	—	—

* The results obtained with the *M. kansasii* reference system are not included since no culture caused deviation phenomena.

† Belonging to cluster 1 but not falling into subclusters 1*a*, 1*b* or 1*c*.

of the 'rhodochrous complex', '*M.*' *rhodochrous*, '*N.*' *pellegrino* and '*N.*' *polychromogenes* (Lind, 1961; Norlin, Lind & Ouchterlony, 1969; Ridell, 1974). In these tests the number of precipitates of the reference system which showed deviation due to the influence of a corresponding precipitinogen in the culture under study were counted. Thus, the total number of precipitates between the well with the antigen under test and the antiserum well was not taken into consideration.

The results of the serological studies were compared with those obtained from the computer analysis of the pooled data (Table 4). Of the 74 cultures belonging to cluster 1, one

had a precipitinogen in common with *M. marianum*, six had one precipitinogen in common with *M. bovis* var. *BCG*, and one of the latter also shared a precipitinogen in common with *M. fortuitum*. None of the remaining 67 cultures had demonstrable precipitinogens in common with the seven mycobacterial reference systems.

Sixty-three of the 74 rhodochrous cultures demonstrated 1 to 5 precipitinogens in common with one or more of the three 'rhodochrous complex' reference systems. It is noteworthy that while 19 of the cultures belonging to subcluster 1*b* had influence upon 2 to 5 of the precipitates of the '*N.*' *polychromogenes* system none of the 37 subcluster 1*a* cultures showed any reaction. A similar division was obtained with the '*M.*' *rhodochrous* reference system, where 23 of the 29 subcluster 1*b* cultures but only four of the subcluster 1*a* cultures influenced two or more precipitates. Similarly, when tested with the Nocardia reference systems the subcluster 1*b* cultures were more reactive (20 out of 29) than those belonging to subcluster 1*a* (9 out of 37). The cluster 1 cultures, therefore, reacted much more frequently in analyses with the Nocardia reference systems (29 cultures) than with the mycobacterial reference systems (7 cultures).

The cultures in clusters 2, 3 and 4, which contained most of the mycobacterial and nocardia marker strains, did not react with the '*N.*' *polychromogenes* or '*M.*' *rhodochrous* reference patterns, and in only two instances reacted with the '*N.*' *pellegrino* system. The two *N. farcinica* cultures had one demonstrable precipitinogen in common with *M. bovis* var. *BCG* and two or three in common with *M. fortuitum*, and culture ATCC25971 had two precipitinogens in common with the *M. avium* reference system. Three of the *Nocardia* cultures, *N. dassonvillei*, *N. madurae* and *N. pelletieri*, had little serological affinity with any of the reference systems, though the latter two cultures had one demonstrable precipitinogen in common with the '*N.*' *pellegrino* reference system.

Chemotaxonomic studies performed by laboratory E. In this laboratory, whole cell hydrolysates were analysed for the presence of the sugars arabinose and galactose, and for *meso*- and LL-diaminopimelic acid (DAP). In addition dried cells were examined to see if they contained true mycolic acids (i.e. containing 80 to 90 carbon atoms) or free nocardomycolic acids (40 to 60 carbon atoms). Nocardomycolic acids were detected using a thin-layer chromatographic method which distinguishes between two of the lipid LCN-A (lipid characteristic of *Nocardia*) analogues, lipid LCN-A type *a* (asteroides) and lipid LCN-A type *c* (calcarea) (Mordarska, Mordarksi & Goodfellow, 1972). LCN-A lipids have properties consistent with those of free nocardomycolic acids (Goodfellow, Minnikin, Patel & Mordarska, 1973).

Correlation of the chemotaxonomic data with the computer-generated clusters and subclusters showed that all cultures in the four clusters contained *meso*-DAP, arabinose and galactose (Table 5). Lipid LCN-A was detected in all the cultures classified in clusters 1 and 3, and true mycolic acids in all the marker cultures of *Mycobacterium*. Subclusters 1*a* and 1*c* could, however, be separated from subcluster 1*b* by the type of lipid LCN-A they contained. Lipid LCN-A type *c* was found in all the subcluster 1*c* cultures and in 30 out of 37 subcluster 1*a* cultures, whereas lipid LCN-A type *a* was mainly confined to cultures classified in subgroup 1*b*. The analogue with the higher R_F value, lipid LCN-A type *a*, was also detected in the Nocardia cultures of cluster 3. The marker cultures labelled *N. dassonvillei*, *N. madurae* and *N. pelletieri* have a cell wall type III and do not contain any type of mycolic acid.

Characters with greatest resolving power for separation of the rhodochrous taxon from Mycobacterium and Nocardia. Eighteen characters were chosen as having the greatest resolving power for the separation of cultures assigned to the four clusters (Table 6). Widely

Table 5. *Distribution of cultures by chemotaxonomy (Laboratory E) and phenetic clustering behaviour*

Cluster	Total no. of cultures	No. of cultures analysed	Arabinose galactose *meso*-DAP	LCN-A type *a*	LCN-A type *c*	Mycolic acids (C80–90)
1*a*	37	33	33	3	30	0
1*b*	29	26	26	24	2	0
1*c*	6	6	6	0	6	0
1*	2	1	1	1	0	0
2	3	3	3	0	0	3
3	3	3	3	3	0	0
4	9	6	6	0	0	6

* Belonging to cluster 1 but not falling into subclusters 1*a*, 1*b* or 1*c*.

Table 6. *Distribution of 18 characters with the greatest resolving power among the four main clusters*

Character	No. positive* (%) Cluster 1 (72)	Cluster 2 (3)	Cluster 3 (3)	Cluster 4 (9)
Staining and colony morphology				
Acid-fast	0	100	0	100
Aerial hyphae	0	0	100	0
Colonies pink, red, orange	94	0	100	11
Growth				
At 10 °C	99	0	100	0
On ethanol as single carbon source	93	0	0	22
On sucrose as single carbon source	100	33	0	22
Lipid analyses				
LCN-A, type *a*	38†	0	100	0
Mycolic acids (C80–90)	0	100	0	100‡
Enzymic activity				
Benzamidase	2	100	100	44
Urease	93	100	100	78
Isonicotinamidase	1	67	100	44
Salicylamidase	1	33	100	33
Allantoinase	0	0	100	0
Succinamidase	1	67	100	44
Malonamidase	1	67	100	33
β-Esterase	50	100	0	78
Growth on 7 % NaCl	71	0	33	0
Susceptibility to 10 i.u. penicillin	86	0	0	0

* No. of cultures analysed is given in parentheses.
† 60 cultures analysed.
‡ 6 cultures analysed.

different taxonomic criteria (staining and colony morphology, growth tests, lipid analyses, enzymic resistance tests) all provide information of diagnostic value.

Characters with greatest resolving power for separation of the subclusters within the rhodochrous taxon (cluster 1). Fourteen characters were chosen as the most useful for the differentiation of the three subclusters of cluster 1 (Table 7). Tyrosine hydrolysis and the lipid LCN tests are particularly useful in distinguishing subclusters 1*a* and 1*b*.

Table 7. *Distribution of* 14 *characters with the greatest resolving power among the three subgroups of the rhodochrous taxon* (*cluster* 1)

Character	No. positive (%) Cluster 1*a* (37)	Cluster 1*b* (29)	Cluster 1*c* (6)
Growth on the following as single carbon source			
Maltose	100	100	0
Mannitol	100	55	0
Sorbitol	100	55	0
Trehalose	92	86	0
2,3-Butylene glycol	100	69	0
m-Hydroxybenzoic acid	70	10	15
Sebacic acid	84	86	0
Sodium lactate	89	21	67
Testosterone	81	7	33
Growth on the following as single carbon/nitrogen source			
Monoethanolamine	43	93	0
Lipid analyses			
LCN-A, type *a*	9†	92‡	0
LCN-A, type *c*	91†	8‡	100
Enzymic activity			
Ferric ammonium citrate uptake	84	69	0
Tyrosine hydrolysis	89	0	100

* No. of cultures analysed is given in parentheses.
† 33 cultures analysed.
‡ 26 cultures analysed.

DISCUSSION

Cultures considered to belong to the 'rhodochrous complex' carry a host of generic and species names and since the late 1950s have challenged taxonomists interested in the actinomycetes. The problem of a generic niche for rhodochrous cultures has exercised investigators interested in the genus *Nocardia* as well as those involved with the genus *Mycobacterium*. Although individual studies carried out in the late 1950s and 1960s furthered our knowledge of the characteristics of the rhodochrous taxon they did not resolve the taxonomic dilemma (Gordon, 1966; Magnusson, 1962; Gordon & Mihm, 1957, 1959). Eventually, several workers independently recognized that the numerical taxonomic procedure was particularly suited to solving taxonomic problems of this nature and, not surprisingly, the results of a number of numerical taxonomic studies have been published during the course of this co-operative project.

Bradley (1971) found that cultures referred to the 'rhodochrous complex' were more closely allied with *N. asteroides* and *N. farcinica* than to *M. tuberculosis*. He also concluded that the rhodochrous taxon was heterogeneous and consisted of at least two clusters, one typified by *N. corallina* and *N. rubra*, the other by *N. erythropolis*. The DNA from strains recovered in the first taxon were found to have 66 to 68 % guanine plus cytosine (GC) whereas those in the latter contained 61 to 63 % GC.

In another numerical taxonomic study, Tsukamura (1971) proposed the genus *Gordona* for a number of 'rhodochrous-like' organisms. However, only six marker cultures of '*M.*' *rhodochrous* were included in this study and none of these clustered with the defined species.

Representatives of the genus *Gordona* were not included in the present study and further work is required to determine the relationship of the genus to the rhodochrous taxon. In a more extensive study (Tsukamura, 1973), 20 '*M.*' *rhodochrous* cultures were included; 10 were classified in a new species *Gordona rhodochroa* and 2 assigned to a renamed taxon *G. rubropertincta*. The ten strains in the former species were all recovered in subcluster 1*a* and the other two in subcluster 1*b*. Tsukamura did, however, concede that the epithet *Gordona* for rhodochrous cultures (Goodfellow, Fleming & Sackin, 1972) might be incorrect.

The most comprehensive numerical taxonomic studies on the 'rhodochrous complex' to date have been presented by Goodfellow (1971) and Goodfellow *et al.* (1972). In the earlier study the rhodochrous taxon was clearly delineated from the genera *Nocardia*, *Actinomadura* and *Oerskovia*, and in the later one a demarcation between the rhodochrous taxon and mycobacteria referred to Runyon's group IV was ascertained. Furthermore, the cluster containing the rhodochrous cultures fell into a number of subclusters, three of which were homogeneous. However, Goodfellow *et al.* (1972) and Cross & Goodfellow (1973) considered that additional evidence was required before the generic status of the '*M*'. *rhodochrous* taxon could be resolved.

Although markedly different test cultures were used, the results of this co-operative study are in good agreement with earlier findings. In all cases the rhodochrous cluster was defined around the 80 % S-level, and the inter-group similarities between the rhodochrous cluster and clusters containing group IV mycobacteria were between 60 and 65 %. Because different rhodochrous cultures were studied it was difficult to compare the subclusters recovered in the various studies. However, subcluster 1*a* (Table 2) can be equated to *N. rubra* (Bradley, 1971), to subcluster 14C (Goodfellow, 1971) and to *G. rhodochroa* (Tsukamura, 1973). Similarly, *N. erythropolis* (Bradley, 1971) appears to be the same taxon as subcluster 14D (Goodfellow, 1971). Subcluster 14A (Goodfellow, 1971), which contained cultures received as *N. pellegrino*, had no counterpart in the co-operative study. Tacquet *et al.* (1971) recovered cultures of '*M.*' *pellegrino* in one of two subclusters in a rhodochrous cluster clearly differentiated from clusters containing mycobacteria and nocardiae.

The results from the individual laboratories yielded clusters in general agreement with those obtained from the analysis of the pooled data. This was even the case with laboratories C and D which examined only 28 and 32 unit characters respectively, a number of characters which would normally be considered insufficient for a numerical taxonomic study. Thus, a wealth of numerical taxonomic data has been obtained which shows that the 'rhodochrous complex' forms a taxon which is distinct both from the genus *Mycobacterium* and from the genus *Nocardia*, and data from independent studies, in general, corroborate this conclusion. The rhodochrous cultures showed much closer serological relationships with the '*M.*' *rhodochrous* and '*N.*' *polychromogenes* reference systems than with those provided by the mycobacterial and nocardia cultures. The serological data also support the classification of the rhodochrous cultures into the two large subclusters 1*a* and 1*b*. The results of the lipid analyses also supported this division, as most of the subcluster 1*b* cultures contained LCN-A type *a*, while those in subclusters 1*a* and 1*c* contained LCN-A type *c*. True mycolic acids are characteristic of mycobacteria, whereas the corresponding nocardomycolic acids were found in *Nocardia sensu stricto* and the rhodochrous taxon. The serological and lipid studies strengthen the conclusions drawn from the numerical taxonomic studies.

The main aim of this study was to determine the taxonomic relationships between the 'rhodochrous complex' and the genera *Mycobacterium* and *Nocardia*. In accordance with some other recent studies (Goodfellow, 1971; Goodfellow *et al.* 1972; Ridell, 1974), the rhodochrous taxon can be distinguished from these taxa, and also from the proposed genus

Actinomadura (comprising *N. dassonvillei*, *N. madurae* and *N. pelletieri*). There is, however, no generally accepted taxonomic niche for the rhodochrous taxon: *Nocardia* (Lechevalier *et al.* 1971), *Gordona* (Tsukamura, 1971) and *Proactinomyces* (Bradley & Bond, 1974) have each been proposed. We believe, however, that the question of a generic location cannot be settled until the relationships of the rhodochrous taxon to the established genera *Arthrobacter* and *Corynebacterium* as well as to *Brevibacterium* – a 'genus *incertae sedis*' – have been ascertained. Similarly, additional studies are required before the taxonomic status of the rhodochrous subclusters can be resolved.

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