Chemical and Numerical Taxonomy of Strains Received as *Gordona aurantiaca*

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Eight strains of *Gordona aurantiaca*, 89 representatives of the genera *Mycobacterium*, *Nocardia* and *Rhodococcus* and a single strain of *Brevibacterium linens* were the subject of numerical phenetic analyses using 92 unit characters. The *G. aurantiaca* cluster was robust in different numerical analyses and equivalent in rank to the genera *Mycobacterium*, *Nocardia* and *Rhodococcus*. The presence of a characteristic mycolic acid and completely unsaturated menaquinones distinguished *G. aurantiaca* strains from mycobacteria, nocardiae and rhodococci. The congruence between the numerical and phenetic data indicate that *G. aurantiaca* strains form a distinct taxon resembling other mycolic acid-containing taxa in wall, fatty acid and polar lipid composition. Since the genus *Gordona* is no longer valid, a new niche is needed for *G. aurantiaca*.

INTRODUCTION

The genus *Gordona* was created for slightly acid-fast organisms found in soil and associated with pulmonary disease (Tsukamura, 1971). As originally proposed, the genus contained *Gordona bronchialis*, *Gordona rubra* and *Gordona terrae* but an additional species, *Gordona aurantiaca*, was added (Tsukamura & Mizuno, 1971) for strains from sputa of patients with cavitary pulmonary tuberculosis or bronchiectasis. Subsequently, Tsukamura (1973, 1974, 1975) suggested that some rhodochrous strains should be classified in the genus and proposed three new species: *Gordona rhodochroa*, *Gordona rosea* and *Gordona rubropertincta* (syn. *G. rubra*). Strains provisionally classified as *Nocardia rubra* were added as *Gordona lentifragmenta* (Tsukamura *et al.*, 1975).

Early phenetic studies indicated the equivocal position of *Gordona aurantiaca* in the genus *Gordona*. *Gordona aurantiaca* strains were found to be only loosely associated with clusters containing other gordonae and showed almost equal similarity to fast-growing mycobacteria (Tsukamura, 1974, 1975). In a subsequent study (Tsukamura *et al.*, 1975), *G. aurantiaca* strains were recovered in a distinct cluster equivalent to those containing fast-growing mycobacteria, nocardiae and other gordonae, respectively, and were distinguished from other gordonae by their β -galactosidase activity and inability to reduce nitrate. The genus *Rhodococcus* was proposed (Goodfellow & Alderson, 1977) for strains classified either in the genus *Gordona* or as members of the 'rhodochrous' complex (Bousfield & Goodfellow, 1976). The type strain of *G. aurantiaca* studied by Goodfellow & Alderson (1977) was only loosely associated with the *Rhodococcus* cluster and it was concluded that additional comparative studies were required to determine the relationships between *G. aurantiaca* and other nocardioform taxa. The assignment of the other *Gordona* species, including the

type species *G. bronchialis*, to *Rhodococcus* leaves *G. aurantiaca* as a taxon in search of a genus.

In the present study *G. aurantiaca* strains were compared with representatives of the genera *Mycobacterium*, *Nocardia* and *Rhodococcus* using numerical and chemotaxonomic methods.

METHODS

Strains. Eight strains of *Gordona aurantiaca*, 10 fast-growing mycobacteria, 13 nocardiae, 65 rhodococci and strains of *Brevibacterium linens* and *Corynebacterium equi* were included in the numerical survey (Table 1). Detailed histories of most of these strains have been given previously (Goodfellow, 1971; Goodfellow *et al.*, 1972; Goodfellow & Alderson, 1977). All of the test strains were maintained on glucose yeast extract agar at room temperature (Gordon & Mihm, 1962).

Collection and coding of data. Strains of *Gordona aurantiaca* (M290 to M296, N663), *Brevibacterium linens* (c40), *Corynebacterium equi* (c36) and *Rhodococcus coprophilus* (N744) were screened for 92 unit characters and the data were added to those previously collected on the remaining strains (Goodfellow & Alderson, 1977).

Computer analysis. Data were analysed using the Clustan 1A program of Wishart (1968) on an IBM370/168 computer using both the simple matching coefficient (S_{SM}) (Sokal & Michener, 1958), which includes positive and negative similarities, and the Jaccard coefficient (S_J) (Sneath, 1957), which excludes negative matches. Clustering was done using both the single linkage and unweighted average linkage (UPGMA) algorithms (Sneath & Sokal, 1973). Data from the S_{SM} and UPGMA analyses were used to prepare a sorted shaded diagram (Fig. 1). Vigour and pattern statistics were calculated (Wishart, 1968) and the data were sorted using the UPGMA algorithm. Mean intragroup and intergroup similarity values were calculated for the clusters defined in the S_{SM} and average linkage analysis.

Lipid analyses. The *G. aurantiaca* strains were grown in shake culture at 30 °C for 3 to 5 d in modified Sauton's medium (Mordarska *et al.*, 1972) supplemented with vitamin B₁ (50 µg ml⁻¹). Cultures were checked for purity at maximum growth, killed by shaking with formalin (1 %, v/v), separated by centrifugation, washed with distilled water and freeze-dried. Dried cells of *Mycobacterium avium* D4, *Rhodococcus* (*Gordona*) *bronchialis* N654, *Rhodococcus corallinus* (*Gordona rubra*) N655 and *Rhodococcus* (*Gordona*) *terrae* N656 (Minnikin *et al.*, 1975; Alshamaony *et al.*, 1976b) were used as standards for the analysis of long-chain components.

Freeze-dried bacteria (50 to 100 mg) were degraded by acid methanolysis and hexane extracts were examined for long-chain components by thin-layer chromatography (t.l.c.) as described previously (Minnikin *et al.*, 1975) using Merck silica gel H (0.5 mm layers) and a developing mixture of petroleum ether (b.p. 60 to 80 °C)/diethyl ether (85:15, v/v). Long-chain components were isolated by preparative t.l.c. on layers (1 mm) of Merck silica gel PF₂₅₄₊₃₆₆, separated bands being detected with ultraviolet light (366 nm).

Long-chain fatty acid methyl esters were analysed using a Perkin-Elmer F11 gas chromatograph fitted with stainless steel columns (200 × 0.4 cm) packed with 3 % ASI 50 cyano propyl silicone (Silar 5CP) on 80 to 100 mesh Gas Chrom Q (Applied Science Laboratories) or 2.5 % OV-1 methyl silicone on 80 to 100 mesh Chromosorb G AW-DMCS (Phase Separations) using nitrogen (40 ml min⁻¹) as carrier gas at isothermal oven temperatures of 165 and 180 °C, respectively. Pyrolysis gas chromatography of mycolic acid methyl esters was done using a short column (50 × 0.4 cm) of the OV-1 packing material with temperature programming at 6 °C min⁻¹ from 120 to 300 °C and the injection heater set at 320 °C.

The isoprenoid quinones were extracted and analysed as described by Collins *et al.* (1977). Mass spectra of the purified menaquinones and mycolic esters were taken on an AEI MS9 instrument using a direct insertion probe, an ionizing voltage of 70 eV and a temperature range of 190 to 220 °C.

Partial mass spectra of the menaquinones have been deposited with the British Library Lending Division, Boston Spa, Yorkshire LS23 7BQ, as Supplementary Publication No. SUP 28008 (3 pages). Copies may be obtained from the BLLD on demand. Where possible, requests should be accompanied by prepaid coupons (held by many university and technical libraries and by the British Council).

RESULTS

Clustering of the strains using the S_{SM} coefficient and the average linkage algorithm

The rhodococci, mycobacteria and nocardiae grouped into three clusters (1, 2 and 3, respectively) defined at the 75 to 80% similarity level (S level) (Fig. 1). The *Gordona aurantiaca* strains were recovered in cluster 4, which was defined at the 87% S level. The single strain of *Brevibacterium linens*, c40, fell outside the four main clusters. The mean

Fig. 1. Shaded diagram based on the analysis of the 98 test strains and 92 unit characters using the S_{SM} coefficient and the average linkage algorithm.

Table 1. Designation and source of strains assigned to clusters 1, 2, 3 and 4 in the S_{8M} , UPGMA analysis

(a) Strains assigned to subcluster 1A		N60	<i>R. erythropolis</i> ; R.E.G., 1293R
N5	<i>Rhodococcus rhodochrous</i> ; NCIB 9701	N63	<i>R. erythropolis</i> ; R.E.G., 463
N27	<i>R. rhodochrous</i> ; NCIB 9160	N65	<i>R. erythropolis</i> ; R.E.G., A7698
N31	<i>R. rhodochrous</i> ; R. E. Gordon (R.E.G.), Rutgers University, U.S.A., w21	N143	<i>R. erythropolis</i> ; M. Turner, Nottingham University, 29
*N54	<i>R. rhodochrous</i> ; ATCC 13808	N148	<i>R. erythropolis</i> ; M. Turner, 83
R10	<i>R. rhodochrous</i> ; ATCC 14341	N209	<i>R. erythropolis</i> ; J. E. Thiemann, Lepetit SpA, Milan, Italy, A2
R15	<i>R. rhodochrous</i> ; ATCC 14350	N146	<i>Rhodococcus rubropertinctus</i> ; M. Turner, 39
R68	<i>R. rhodochrous</i> ; ATCC 25726	(g) Strains assigned to subcluster 1I	
R73	<i>R. rhodochrous</i> ; ATCC 25731	N443	<i>Rhodococcus rhodnii</i> ; P. Hill, Edinburgh University, A1
R74	<i>R. rhodochrous</i> ; ATCC 25732	N444	<i>R. rhodnii</i> ; P. Hill, A/0
R79	<i>R. rhodochrous</i> ; ATCC 25737	*N445	<i>R. rhodnii</i> ; P. Hill, B/0
(b) Strains assigned to subcluster 1C		N446	<i>R. rhodnii</i> ; P. Hill, B/1
*N654	<i>Rhodococcus bronchialis</i> ; NCTC 10667	(h) Strains assigned to subcluster 1J	
N450	<i>Rhodococcus corallinus</i> , R. Bönicke, Institut für Experimentelle Biologie und Medizin, Borstel, West Germany, SN5303	N650	<i>Rhodococcus coprophilus</i> ; T. J. Rowbotham, Bradford University, CUB628.
N451	<i>R. corallinus</i> ; R. Bönicke, SN5302	N651	<i>R. coprophilus</i> ; T. J. Rowbotham, CUB118
*N655	<i>R. corallinus</i> ; NCTC 10668	N652	<i>R. coprophilus</i> ; T. J. Rowbotham, N38
*N657	<i>R. corallinus</i> ; NCTC 10668	N653	<i>R. coprophilus</i> ; T. J. Rowbotham, N19
N658	<i>R. corallinus</i> ; ATCC 60004	*N744	<i>R. coprophilus</i> ; NCIB 11211
*N656	<i>Rhodococcus terrae</i> ; NCTC 10669	(i) Strains assigned to cluster 1	
*N659	<i>R. terrae</i> ; NCTC 10669	N647	<i>Nocardia kiryani</i> ; G. Michel, Lyon University, France, IM1374
*N662	<i>R. terrae</i> ; H. Mordarska, Hirsfeld, Institute, Wrocław, Poland, r6	*N660	<i>Rhodococcus bronchialis</i> ; NCTC 10667
(c) Strains assigned to subcluster 1D		(j) Strains assigned to cluster 2	
N111	<i>Rhodococcus rhodochrous</i> ; R.E.G., 562	*M12	<i>Mycobacterium aurum</i> ; ATCC 23366
*N361	<i>Rhodococcus ruber</i> ; M. Tsukamura, Chuba Chest Hospital, Obu, Aichi-ken 474, Japan, M-1	*M89	<i>Mycobacterium borstelense</i> ; ATCC 19235
N363	<i>R. ruber</i> ; M. Tsukamura, M-192	*M158	<i>Mycobacterium diernhoferi</i> ; ATCC 19340
N420	<i>R. ruber</i> ; A. Tacquet, Pasteur Institute, Lille, 906	*M3	<i>Mycobacterium flavescens</i> ; ATCC 14474
N422	<i>R. ruber</i> ; A. Tacquet, 107	*M93	<i>Mycobacterium fortuitum</i> ; ATCC 6841
N448	<i>R. ruber</i> ; R. Bönicke, SN5104	*M17	<i>Mycobacterium parafortuitum</i> ; ATCC 19686
N455	<i>R. ruber</i> ; R. Bönicke, SN5203	*M6	<i>Mycobacterium peregrinum</i> ; ATCC 23023
(d) Strains assigned to subcluster 1F		*M98	<i>Mycobacterium phlei</i> ; ATCC 11758
*C36	<i>Corynebacterium equi</i> ; NCTC 1621	*M101	<i>Mycobacterium smegmatis</i> ; ATCC 14468
R39	<i>Rhodococcus equi</i> ; ATCC 25694	*M11	<i>Mycobacterium thermoresistibile</i> ; ATCC 19527
R59	<i>R. equi</i> ; ATCC 25715	(k) Strains assigned to cluster 3	
R60	<i>R. equi</i> ; ATCC 25716	N13	<i>Nocardia asteroides</i> ; NCTC 8595
R70	<i>R. equi</i> ; ATCC 25728	N100	<i>N. asteroides</i> ; R.E.G., 652
R71	<i>R. equi</i> ; ATCC 25729	N105	<i>N. asteroides</i> ; R.E.G., 9969
R76	<i>R. equi</i> ; ATCC 25734	N233	<i>N. asteroides</i> ; R. Olds, Cambridge University, CN750
(e) Strains assigned to subcluster 1G		*N317	<i>N. asteroides</i> ; ATCC 19247
*N4	<i>Rhodococcus rubropertinctus</i> ; NCIB 9664	N14	<i>Nocardia brasiliensis</i> ; NCTC 10300
N113	<i>R. rubropertinctus</i> ; V. B. D. Skerman, Queensland University, Brisbane, Australia, 134	N48	<i>N. brasiliensis</i> ; R.E.G., 744
R17	<i>R. rubropertinctus</i> ; ATCC 25669	N311	<i>N. brasiliensis</i> ; F. Mariat, Institut Pasteur, Paris, 768
R21	<i>R. rubropertinctus</i> ; ATCC 25673	N312	<i>N. brasiliensis</i> ; F. Mariat, 337
R24	<i>R. rubropertinctus</i> ; ATCC 25676	*N318	<i>N. brasiliensis</i> ; ATCC 19296
R34	<i>R. rubropertinctus</i> ; ATCC 25688	*N36	<i>Nocardia caviae</i> ; NCTC 1934
R43	<i>R. rubropertinctus</i> ; ATCC 25699	N231	<i>N. caviae</i> ; R. Olds, CN749
R55	<i>R. rubropertinctus</i> ; ATCC 25710	N313	<i>N. caviae</i> ; F. Mariat, 751
R56	<i>R. rubropertinctus</i> ; ATCC 25711	(l) Strains assigned to cluster 4	
R58	<i>R. rubropertinctus</i> ; ATCC 25714	*M290	<i>Gordona aurantiaca</i> ; NCTC 10741
(f) Strains assigned to subcluster 1H		M291 to M296	<i>G. aurantiaca</i> ; M. Tsukamura, 3477, 4409, 4465, 7972, 7973, 8128
*N11	<i>Rhodococcus erythropolis</i> ; NCIB 9158	*N663	<i>G. aurantiaca</i> ; NCTC 10741
N12	<i>R. erythropolis</i> ; NCIB 9159	(m) Strain not assigned to any cluster	
N23	<i>R. erythropolis</i> ; CCM 227	c40	<i>Brevibacterium linens</i> ; ATCC 9174
N57	<i>R. erythropolis</i> ; R.E.G., 1293s		
N58	<i>R. erythropolis</i> ; R.E.G., 1257		

* Type strains.

M, N, R, Laboratory numbers; ATCC, American Type Culture Collection; CCM, Czechoslovak Collection of Microorganisms; NCIB, National Collection of Industrial Bacteria; NCTC, National Collection of Type Cultures.

Table 2. *Percentage intergroup similarities between the Gordona aurantiaca cluster and the other clusters and subclusters obtained from the S_{SM} , average linkage analysis*

Phenon	Taxon	Percentage similarity to <i>G. aurantiaca</i>
1	<i>Rhodococcus</i>	68.1
2	<i>Mycobacterium</i>	62.9
3	<i>Nocardia</i>	54.0
1G	<i>Rhodococcus rubropertinctus</i>	71.6
1A	<i>Rhodococcus rhodochrous</i>	70.7
1H	<i>Rhodococcus erythropolis</i>	70.4
1I	<i>Rhodococcus rhodnii</i>	68.1
1C	<i>Rhodococcus bronchialis</i>	67.4
	<i>Rhodococcus corallinus</i>	
	<i>Rhodococcus terrae</i>	
1D	<i>Rhodococcus ruber</i>	66.1
1F	<i>Rhodococcus equi</i>	63.5
1J	<i>Rhodococcus coprophilus</i>	58.3
3b	<i>Nocardia caviae</i>	59.5
3c	<i>Nocardia brasiliensis</i>	55.4
3a	<i>Nocardia asteroides</i>	48.1
—	<i>Brevibacterium linens</i>	69.0

intragroup S value for the *G. aurantiaca* cluster was 91.5%; the mean intergroup similarities between it and the other clusters are shown in Table 2. Mean intragroup S values for the *Rhodococcus* cluster and its subclusters, the *Mycobacterium* cluster and the *Nocardia* cluster were determined by Goodfellow & Alderson (1977).

Cluster 1 (*Rhodococcus*) was divided into nine subclusters of essentially similar composition to those obtained by Goodfellow & Alderson (1977) who included 80 additional rhodococci in their analyses. These authors attributed the occurrence of *Rhodococcus ruber* strains in two subclusters, 1D and 1E, to sampling error. The strains of subcluster 1E were not, therefore, included in the present analysis. The single representative of Goodfellow & Alderson's (1977) subcluster 1B (N31) fell within subcluster 1A, a result in good accord with DNA:DNA reassociation data (Mordarski *et al.*, 1977). As expected the type strains of *Corynebacterium equi* (c36) and *Rhodococcus coprophilus* were recovered in the subclusters bearing these names (1F and 1J, respectively).

Clustering of the strains using other coefficients and clustering algorithms

The four major clusters were recovered in the three other analyses. Use of the S_j coefficient had a marginal effect on the composition of the *Rhodococcus* subclusters but the main clusters were well separated. With the exception of subcluster 1A (*Rhodococcus rhodochrous* strains), which was ill-defined, use of the single linkage algorithm had little effect on the composition of subclusters, which remained distinct.

The pattern coefficient seeks to allow for differences in growth rates, incubation periods and similar factors which might contribute to overall dissimilarity between two strains. It excludes the vigour component (D_v), which expresses the difference in total metabolic activity of two strains, from the total difference between the strains, leaving clusters defined on the basis of pattern difference (D_p) (Sneath, 1968). The removal of the vigour component from total dissimilarities did not have any effect on the distribution of strains to the four main clusters and only marginally affected the clustering of the rhodococci, members of subcluster 1D (*Rhodococcus ruber*) falling within subcluster 1C (*Rhodococcus bronchialis*, *Rhodococcus corallinus*, *Rhodococcus terrae*). Though well separated from the other three clusters in the pattern analysis, the *Gordona aurantiaca* cluster was joined by the marker strain *Mycobacterium borstelense* (m89) and less strongly by *Brevibacterium linens* (c40).

Table 3. *Percentage frequencies of positive characters found in the major clusters*

Character	Cluster			
	1 <i>Rhodococcus</i> (66)*	2 <i>Myco- bacterium</i> (10)	3 <i>Nocardia</i> (13)	4 <i>Gordona aurantiaca</i> (8)
Staining and colony morphology:				
Strongly acid-fast	0	100	0	25
Aerial hyphae, sparse	20	0	92	0
Aerial hyphae, moderate	1	0	85	0
Aerial hyphae, abundant	0	0	77	0
Colonies white	17	70	23	100
Colonies pink, red, orange	83	10	77	0
Colonies yellow	0	20	0	0
Diffusible exopigment	0	0	100	0
Elevation convex	70	100	8	100
Elevation irregular	8	0	92	0
Margin entire	65	100	0	100
Margin filamentous	22	0	92	0
Mycelium formed	97	0	100	0
Degradation of:				
Adenine	39	0	0	0
Allantoin, weak	15	0	100	63
Allantoin, strong	0	0	23	50
Casein	0	0	38	0
Elastin	0	0	38	0
Hypoxanthine	0	0	61	75
Tween 20	100	100	69	100
Tween 40	97	100	54	100
Tween 60	98	100	54	100
Tyrosine	46	0	38	75
Urea, weak	82	50	100	88
Urea, strong	49	0	100	75
Xanthine	0	0	23	50
Growth on sole carbon source (at 1 g l ⁻¹):				
Adonitol	0	50	0	0
L-Arabinose	3	50	15	0
Cellobiose	0	0	0	38
D-Fructose	97	80	92	100
D-Galactose	3	10	38	100
Glycerol	87	70	100	100
Growth on sole carbon/nitrogen source:				
Acetamide	48	30	46	88
Serine	3	20	38	100
Trimethylenediamine	0	60	0	0
Growth at:				
10 °C	65	0	23	100
40 °C	94	70	92	0
45 °C	10	30	38	0
50 °C	1	20	15	0
Growth in the presence of:				
0.0001 % (w/v) Crystal violet	91	100	23	100
0.001 % (w/v) Crystal violet	60	50	8	100
0.01 % (w/v) Phenol	100	100	100	100
0.1 % (w/v) Phenol	73	0	38	100
0.02 % (v/v) Phenylethanol	100	100	54	100
0.03 % (v/v) Phenylethanol	69	0	23	100
0.01 % (w/v) Sodium azide	60	10	31	75
0.02 % (w/v) Sodium azide	52	0	0	38
5.0 % (w/v) Sodium chloride	97	70	85	100
7.0 % (w/v) Sodium chloride	83	0	31	100
Susceptibility to 10 i.u. penicillin	87	0	0	13
Enzyme activity:				
Nitrate reduction	96	90	100	25
<i>m</i> -Nitrophenoloxidase	8	0	0	0
<i>p</i> -Nitrophenoloxidase	10	0	0	0
Lipid LCN-A	100	100	100	0

* Number of cultures analysed is given in parentheses.
None of the strains used raffinose as sole carbon source.

Table 4. *Percentage frequencies of positive characters found in the Rhodococcus subclusters and the Gordona aurantiaca cluster*

Character	Cluster/subcluster								
	1A, B (10)*	1C (9)	1D (7)	1F (7)	1G (10)	1H (12)	1I (4)	1J (5)	4 (8)
Staining and colony morphology:									
Strongly acid-fast	0	0	0	0	0	0	0	0	25
Aerial hyphae, sparse	10	0	29	0	10	33	0	20	0
Aerial hyphae, moderate	0	0	14	0	0	0	0	0	0
Aerial hyphae, abundant	0	0	0	0	0	0	25	0	0
Colonies white	0	0	14	29	30	50	25	0	100
Colonies pink, red, orange	100	100	86	71	70	50	75	100	0
Elevation convex	90	56	100	100	100	83	100	20	100
Elevation irregular	0	0	0	0	0	17	0	80	0
Margin entire	90	33	29	100	100	100	100	0	100
Margin filamentous	10	11	71	0	0	0	0	100	0
Mycelium formed	100	44	100	86	100	100	100	80	0
Degradation of:									
Adenine	70	0	14	86	0	92	0	20	0
Allantoin, weak	0	0	0	0	10	92	0	0	63
Allantoin, strong	0	0	0	0	0	0	0	0	50
Hypoxanthine	0	0	0	0	0	0	0	0	75
Tween 40	100	100	100	100	100	100	50	100	100
Tween 60	100	100	100	100	100	100	100	80	100
Tyrosine	90	0	86	0	0	75	100	0	75
Urea, weak	60	88	71	100	100	92	100	80	88
Urea, strong	50	11	0	71	90	92	50	0	75
Xanthine	0	0	0	0	0	0	0	0	50
Growth on sole carbon source (at 1 g l⁻¹):									
Adonitol	0	0	0	0	0	0	0	0	0
L-Arabinose	0	0	0	0	0	8	25	0	0
Cellobiose	0	0	0	0	0	0	0	0	38
D-Fructose	100	100	100	71	100	100	100	100	100
Growth on sole carbon/nitrogen source:									
Acetamide	40	33	86	48	40	67	100	0	88
Serine	0	0	0	0	0	0	0	0	100
Growth at:									
10 °C	90	0	29	100	90	83	25	40	100
40 °C	100	100	100	100	100	100	25	80	0
45 °C	0	0	86	0	0	8	0	0	0
50 °C	0	0	0	0	0	8	0	0	0
Growth in the presence of:									
0.0001 % (w/v) Crystal violet	90	100	100	100	100	58	100	100	100
0.001 % (w/v) Crystal violet	50	100	100	86	60	8	75	100	100
0.1 % (w/v) Phenol	100	100	100	29	80	33	25	100	100
0.03 % (v/v) Phenylethanol	90	100	100	57	70	33	25	80	100
0.01 % (w/v) Sodium azide	10	100	86	86	70	67	50	40	75
0.02 % (w/v) Sodium azide	20	89	100	43	20	67	75	20	38
5.0 % (w/v) Sodium chloride	90	100	100	100	90	100	100	100	100
7.0 % (w/v) Sodium chloride	50	100	100	43	90	83	100	100	100
Susceptibility to 10 i.u. penicillin	100	33	100	57	100	100	100	100	13
Enzyme activity:									
Nitrate reduction	100	78	100	100	100	92	100	100	25
m-Nitrophenoloxidase	60	11	14	0	0	0	0	0	0
p-Nitrophenoloxidase	0	11	0	14	0	0	0	0	0
Lipid LCN-A	100	100	100	100	100	100	100	100	0

* Number of cultures analysed is given in parentheses.

None of the strains produced yellow colonies or diffusible pigment, degraded casein or elastin, utilized lactose or raffinose as sole carbon source, or utilized trimethylenediamine as sole carbon and nitrogen source. All degraded Tween 20, utilized sodium acetate as sole carbon source and grew in the presence of 0.01 % (w/v) phenol and 0.02 % (w/v) phenylethanol.

Table 5. *Distribution of characters with the greatest resolving power for differentiating Gordona aurantiaca, Rhodococcus, Mycobacterium and Nocardia*

Results indicate the percentage frequency of positive characters.

Character	Cluster			
	1 <i>Rhodococcus</i> (66)*	2 <i>Myco- bacterium</i> (10)	3 <i>Nocardia</i> (13)	4 <i>Gordona aurantiaca</i> (8)
Staining and colony morphology:				
Strongly acid-fast	0	100	0	25
Mycelium formed	97	0	100	0
Aerial hyphae formed	20	0	92	0
Margin entire	65	100	0	100
Colonies pink, red, orange	83	10	77	0
Diffusible exopigment	0	0	100	0
Growth on sole carbon source (at 1 g l ⁻¹):				
Ethanol	97	20	0	100
Galactose	3	10	38	100
Inulin	90	80	8	0
Melezitose	8	0	0	100
Xylose	3	50	15	100
Growth at 40 °C	94	70	92	0
Growth in the presence of:				
7.0 % (w/v) Sodium chloride	83	0	31	100
Susceptibility to 10 i.u. penicillin	87	0	0	13

* Number of cultures analysed is given in parentheses.

These latter findings were considered to be artefacts of the pattern coefficient (see Discussion).

Reproducibility of results

Discrepancies between the data of Goodfellow & Alderson (1977) and those from the present study were assessed. *Gordona aurantiaca* strain N663 was included in both studies; the test error between these duplicate strains was approximately 6.5%. In the present study, *G. aurantiaca* strains M290 and N663 were duplicates with an S_{SM} value of 97.9% corresponding to a probability of error, P , of 1%.

Differentiation between *Gordona aurantiaca* and the other taxa

The percentages of positive responses to each test within the clusters and subclusters are shown in Tables 3 and 4. Fourteen characters were selected as having the greatest resolving power for the differentiation of the four major clusters (Table 5).

Lipid analyses

Thin-layer chromatographic analysis of whole-organism methanolsates of the *G. aurantiaca* strains showed the presence of two major components (Fig. 2). The most mobile fractions ($R_F > 0.6$) corresponded to methyl esters of long-chain non-hydroxylated fatty acids and the least mobile components were similar in their migration to methyl esters of mycolic acids (Minnikin *et al.*, 1975). The supposed mycolic esters from *G. aurantiaca* strains were more mobile on t.l.c. than were the methyl mycolates from the *Rhodococcus* strains and showed similar mobility to the least polar mycolic ester from *Mycobacterium avium*.

Gas chromatographic analysis of the long-chain fatty acid esters of *G. aurantiaca* strains

Fig. 2. Thin-layer chromatography of whole-organism methanolysates of *Gordona aurantiaca* and related organisms.

(Table 6) revealed major proportions of straight-chain saturated and unsaturated acids and a component co-chromatographing with methyl 10-methyloctadecanoate (tuberculostearate) from *Mycobacterium avium*. Methyl tuberculostearate was not separated from methyl octadecenoate on the polar (Silar 5CP) stationary phase.

The long-chain components from *G. aurantiaca* strains which were chromatographically similar to mycolic esters (Fig. 2) all gave essentially the same results on pyrolysis gas chromatography and mass spectrometry. The mycolic ester from *G. aurantiaca* M290, for example, produced, on pyrolysis gas chromatography, four peaks of comparable intensity corresponding to straight-chain C_{20} and C_{22} saturated and mono-unsaturated esters. Mass spectrometry (Etémadi, 1967; Alshamaony *et al.*, 1976*a*) of the same ester showed medium-sized peaks at m/e 326 and 354 corresponding to the C_{20} and C_{22} saturated acids released on pyrolysis. No significant fragments corresponding to the unsaturated components (m/e 324 and 352) were observed; loss of the elements of methanol (32 mass units) apparently gave rise to medium-sized peaks at m/e 292 and 320. A complex series of fragments corresponding to aldehydes formed by pyrolysis was seen at the following m/e values (main component in bold type; figures in parentheses specify the number of carbon atoms and degree of unsaturation): 684 ($C_{48:2}$), 686 ($C_{48:1}$), 710 ($C_{50:3}$), **712** ($C_{50:2}$), 734 ($C_{52:5}$), 736 ($C_{52:4}$), 738 ($C_{52:3}$), 740 ($C_{52:2}$), 760 ($C_{54:6}$), 762 ($C_{54:5}$), 764 ($C_{54:4}$), 766 ($C_{54:3}$), 788 ($C_{56:6}$) and 790

Table 6. *Fatty acid composition of Gordona aurantiaca strains, determined by gas chromatography of methyl esters on polar and non-polar columns*

Strain	Relative percentages (w/w) of fatty acids*									
	14:0	15:0	14:1	16:0	16:1	17:0	17:1	18:0	18:1	t-19
<i>G. aurantiaca</i> M290	1.3	—	—	65.7	2.3	—	—	14.9	6.0	9.8
<i>G. aurantiaca</i> M291	4.0	1.0	0.9	41.9	4.3	0.4	0.4	12.5	33.6	1.0
<i>G. aurantiaca</i> M292	4.6	—	—	43.4	7.0	—	—	4.4	24.3	16.3
<i>G. aurantiaca</i> M293	2.2	—	—	34.5	6.5	—	—	17.7	18.2	20.9
<i>G. aurantiaca</i> M294	3.0	0.5	—	41.2	5.5	—	—	3.4	25.9	20.5
<i>G. aurantiaca</i> M295	3.0	0.2	—	39.5	6.4	—	—	3.3	27.2	20.4
<i>G. aurantiaca</i> M296	3.4	—	—	40.0	5.6	—	—	3.5	22.6	24.9

* The number preceding the colon indicates the number of carbon atoms and the number after the colon indicates the number of double bonds; t-19 denotes 10-methyloctadecanoic (tuberculostearic) acid.

Table 7. *Peaks corresponding to molecular ions in the mass spectra of menaquinones isolated from strains of Gordona aurantiaca*

The main component of each series is denoted by + + +, any components greater than 50 % of the main peak by + + and all other significant components by +.

Menaquinone isoprenologue <i>m/e</i>	MK-7 648	MK-8 716	MK-9 784	MK-10 852
<i>G. aurantiaca</i> M290	+	+	+++	—
<i>G. aurantiaca</i> M291	+	++	+++	—
<i>G. aurantiaca</i> M292	+	+	+++	—
<i>G. aurantiaca</i> M293	+	+	+++	—
<i>G. aurantiaca</i> M294	+	+	+++	—
<i>G. aurantiaca</i> M295	+	+	+++	+
<i>G. aurantiaca</i> M296	+	+	+++	+

(C_{56:5}). In the higher mass range, fragments corresponding to anhydromycolates, formed by elimination of a molecule of water, were found at *m/e* 992 (C_{68:2}), 994 (C_{68:1}), 1020 (C_{70:2}), 1042 (C_{72:5}), 1044 (C_{72:4}), 1046 (C_{72:3}), 1048 (C_{72:2}), 1070 (C_{74:5}) and 1072 (C_{74:4}).

Extracts of the test strains contained menaquinones which co-chromatographed on t.l.c. with vitamin K. The most significant peak in the spectra occurred at *m/e* 784 corresponding to an unsaturated menaquinone with nine isoprene units, abbreviated as MK-9; smaller peaks at *m/e* 648, 716 and 852 correspond to the minor components MK-7, MK-8 and MK-10 (Table 7).

DISCUSSION

Consistency between classifications based on different taxonomic techniques and methods is a pre-requisite for a stable taxonomy. Thus, numerical taxonomy can establish clusters whose robustness can be tested using different similarity coefficients and clustering algorithms (Austin & Colwell, 1977), while chemotaxonomy seeks to highlight stable and discontinuously distributed characters that can be used as markers in identification.

In the present study, the *G. aurantiaca* strains formed a distinct cluster with all of the similarity coefficients and algorithms tested and, with few exceptions, the numerical classification of the other strains confirmed previous findings (Goodfellow & Alderson, 1977). The effect of the exclusion of the 80 rhodococci examined in the earlier analyses was to reduce the tightness of some of the *Rhodococcus* subclusters established using the two clustering algorithms. The rhodococci included in the present study were selected to represent the whole range of variation found in the *Rhodococcus* taxon (Goodfellow & Alderson, 1977), some being taken from the edges of subclusters thereby increasing the

likelihood of peripheral members joining neighbouring subclusters in analyses based on a smaller sample of strains. This sampling problem was evident in the case of the *Rhodococcus rhodochromis* subcluster (1A) which had been defined by 49 strains (Goodfellow & Alderson, 1977) of which only 10 were included in the present study; not all of these strains fell into a well defined subcluster in all of the numerical analyses.

The clustering of *Mycobacterium borstelense* (M89) and *Brevibacterium linens* (C40) with the *G. aurantiaca* strains in the pattern difference analysis is considered to be a consequence of the very large difference in vigour between these organisms. Thus, while the *G. aurantiaca* strains gave some 55 positive test responses, *M. borstelense* was coded for only 22 positive characters. The pattern difference coefficient calculates the product of the number of tests for which one organism gives a positive response and the other gives a negative one and the number for the reciprocal situation. Where the many positive responses of the vigorous organism coincide with the few of the less vigorous one, this product will be small giving an artificially small pattern difference. This masking effect on the pattern difference coefficient can be detected by comparing the relative numbers of positive tests given by each strain and confirmed by reference to the S_{SM} or S_J coefficients.

In light of these considerations and the agreement between the various numerical analyses, the *Gordona aurantiaca* cluster is considered to be a distinct and robust grouping, equal in rank to the clusters corresponding to the genera *Mycobacterium*, *Nocardia* and *Rhodococcus*. There was sufficient consistency within clusters and differences between them for some characters to be provisionally weighted for identification (Table 5). Neither *G. aurantiaca* nor any other of the test strains showed a high overall similarity with the single strain of *Brevibacterium linens*.

The type strain of *G. aurantiaca* contains meso-diaminopimelic acid, arabinose and galactose in its walls (I.J. Bousfield, personal communication) and the taxon can, therefore, be provisionally added to the list of genera with a wall chemotype IV (Lechevalier, 1976; Goodfellow & Minnikin, 1977). All of the *G. aurantiaca* strains contained characteristic highly unsaturated mycolic acids centred around C_{70} which released C_{20} and C_{22} straight-chain saturated and unsaturated acid derivatives on pyrolysis. Further detailed studies are needed before the exact structures of these mycolic acids can be established but the present data distinguish them from previously described acids. The mycolic acids from mycobacteria usually release C_{22} to C_{26} completely saturated acids on pyrolysis (Lechevalier *et al.*, 1973); they are complex mixtures of acids having other oxygen functions in addition to the hydroxy acid unit and, to date, have never been found to contain more than two points of unsaturation in the molecule (Etémadi, 1967; Minnikin *et al.*, 1975). Mycolic acids from true corynebacteria, nocardiae, rhodococci and related organisms release, on pyrolysis, acids in the range C_{12} to C_{18} (Lechevalier *et al.*, 1973) and, in general, contain less than 70 carbon atoms (Minnikin & Goodfellow, 1976). Unsaturation in the chain in 2-position of mycolates is also unusual but has been found in the mycolic acids from *Nocardia amarae* (Lechevalier & Lechevalier, 1974) and certain other individual strains (Minnikin & Goodfellow, 1976). The non-hydroxylated fatty acid composition of *G. aurantiaca* strains, containing straight-chain saturated and unsaturated acids and tuberculostearic acid, is similar to that of mycolic acid-containing taxa such as *Mycobacterium*, *Nocardia* and *Rhodococcus* (Kroppenstedt & Kützner, 1976, 1978).

The presence of fully unsaturated menaquinones having nine isoprene units (MK-9) distinguishes *G. aurantiaca* strains from other mycolic acid-containing taxa which usually have dihydrogenated menaquinones (Yamada *et al.*, 1976; Collins *et al.*, 1977). The polar lipids of the type strain of *G. aurantiaca*, examined previously (Minnikin *et al.*, 1977), were similar in general pattern to those of a variety of other mycolic acid-containing bacteria, containing significant amounts of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and mono- and diacylated phosphatidylinositol mannosides. The presence of phosphatidylethanolamine does, however, distinguish *G. aurantiaca*

strains from corynebacteria, and *Rhodococcus bronchialis*, *R. ruber* and *R. terrae*, all formerly labelled *Gordona*, were found not to contain diacylated phosphatidylinositol mannosides (Minnikin *et al.*, 1977).

Thus *G. aurantiaca* resembles *Mycobacterium*, *Nocardia* and *Rhodococcus* on the basis of wall, fatty acid and polar lipid composition but, on evidence from numerical phenetic, isoprenoid quinone and mycolic acid analysis, can be clearly distinguished from them. The inclusion of all of the other *Gordona* species in the genus *Rhodococcus* (Goodfellow & Alderson, 1977) makes the genus *Gordona* invalid and consequently a new taxonomic niche is required for *G. aurantiaca*. On balance, the evidence presently available favours raising the taxon to generic status but further studies on additional strains are required if such a proposal is to be made with confidence. Until these additional studies have been done the organisms should be provisionally named the '*aurantiaca*' taxon.

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