The Actinomycete-genus *Rhodococcus*: A Home for the 'rhodochrous' Complex

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SUMMARY

A numerical taxonomic classification study was carried out on 177 strains representing the 'rhodochrous' complex and the genera *Gordona*, *Mycobacterium* and *Nocardia*. The strains were examined for 92 unit characters and the data were analysed by computer. Three clusters were defined at the 75 to 80 % similarity level. The first was a heterogeneous cluster corresponding to the 'rhodochrous' taxon whereas the other two contained *Mycobacterium* and *Nocardia* strains respectively. The good correlation between the numerical analysis and chemotaxonomic, serological and genetical data collected from previous studies provides sufficient evidence for raising the 'rhodochrous' taxon to generic status. We consider the generic name *Rhodococcus* Zopf to have priority over *Proactinomyces* (Jensen) Bradley & Bond, *Jensenia* Bisset & Moore and *Gordona* Tsukamura. In addition to the type species, *Rhodococcus rhodochrous*, nine species are recognized: *R. bronchialis*, *R. coprophilus*, *R. corallinus*, *R. erythropolis*, *R. equi*, *R. rhodnii*, *R. rubrus*, *R. rubropertinctus* and *R. terrae*.

INTRODUCTION

The taxonomic history of bacteria classified variously as *Mycobacterium rhodochrous* (Overbeck, 1891; Gordon & Mihm, 1957), the 'rhodochrous' complex (Bradley, 1971), the 'rhodochrous' taxon (Goodfellow *et al.*, 1974) or the *M. rhodochrous* complex (Cross & Rowbotham, 1974) has been traced by Bousfield & Goodfellow (1976). Gordon & Mihm (1957) proposed the taxon *Mycobacterium rhodochrous* for a group of similar strains which they had received under a plethora of generic and specific names, the oldest being *Rhodococcus rhodochrous* Overbeck 1891; Zopf 1891. In subsequent studies (Gordon & Mihm, 1959, 1961; Gordon, 1966) further nomenclatural species were reduced to synonyms of *M. rhodochrous* and a more comprehensive description of the taxon was given. Gordon (1966) noted some variation in rhodochrous strains but nevertheless described them as a good species in search of a genus; *Nocardia*, *Mycobacterium* and *Corynebacterium* were offered as possible niches. However, in the eighth edition of *Bergey's Manual of Determinative Bacteriology*, bacteria formerly assigned to *M. rhodochrous* are scattered amongst species of *Nocardia* with the proviso that further work was needed to clarify their taxonomic position (McClung, 1974; Runyon, Wayne & Kubica, 1974).

The 'rhodochrous' complex forms a recognizable but heterogeneous taxon and can be distinguished from *Mycobacterium* and *Nocardia* on the basis of antibiotic sensitivity (Goodfellow & Orchard, 1974), chemical (Ratledge & Patel, 1976*a, b*), DNA reassociation (Bradley & Mordarski, 1976; Mordarski *et al.*, 1976, 1977), numerical phenetic (Goodfellow *et al.*, 1974; Bousfield & Goodfellow, 1976), serological (Ridell & Norlin, 1973;

Lind & Ridell, 1976) and phage-susceptibility studies (Manion *et al.*, 1964; Jones & Bradley, 1964; Pietkiewicz *et al.*, 1974). There is, however, little agreement on the internal structure of the taxon and comparisons between different studies are hampered by the paucity of extant type cultures and the questionable identification of many strains.

The heterogeneity of the '*rhodochrous*' complex is most clearly seen from numerical phenetic studies (Bradley, 1971; Goodfellow, 1971; Tacquet *et al.*, 1971; Goodfellow *et al.*, 1974; Jones, 1975) but is also apparent from chemical (Goodfellow *et al.*, 1974; Collins *et al.*, 1977; Alshamaony *et al.*, 1976*a, b*), DNA:DNA pairing (Clark, Brownell & Bradley, 1971; Bradley, 1973; Bradley & Mordarski, 1976; Mordarski *et al.*, 1976, 1977), physiological (Gordon, 1966; Lechevalier & Lechevalier, 1974; Mordarska, Mordarski & Pietkiewicz, 1973), serological (Magnusson, 1962; Ridell, 1974; Lind & Ridell, 1976), genetical and actinophage studies (Brownell, Adams & Bradley, 1967; Adams, Adams & Brownell, 1970). Since few strains are common to all of these investigations it is difficult to determine whether or not, and to what extent, defined groups overlap. The taxonomic situation is further confused by the placing of some strains, similar to rhodochrous bacteria, in the genus *Gordona* (Tsukamura, 1971). This genus is listed as *incertae sedis* in the current edition of *Bergey's Manual of Determinative Bacteriology* (McClung, 1974).

As originally proposed, the genus *Gordona* contained the species *Gordona bronchialis*, *G. rubra* and *G. terrae* (Tsukamura, 1971). A further species, *G. aurantiaca*, was described by Tsukamura & Mizuno (1971). Subsequently, Tsukamura (1973, 1974, 1975) suggested that some rhodochrous strains should also be classified in the genus and proposed three new species: *G. rhodochroa*, *G. rosea* and *G. rubropertincta* (syn. *G. rubra*). Strains provisionally classified as *Nocardia rubra* were also included in the genus as *G. lentifragmenta* (Tsukamura, Mizuno & Murata, 1975). The enlarged genus *Gordona* was distinguished from *Nocardia* and most *Mycobacterium* taxa in a comprehensive numerical phenetic analysis (Tsukamura *et al.*, 1975).

Bousfield & Goodfellow (1976) discussed the taxonomic status of strains of the '*rhodochrous*' complex and suggested that they might be classified as *Mycobacterium rhodochrous*, or reclassified in an alternative established genus or considered as a genus in their own right. Possible generic names, cautiously intimated by Cross & Goodfellow (1973), were *Rhodococcus* Zopf 1891, *Proactinomyces* (Jensen 1931) Bradley & Bond 1974, *Jensenia* Bisset & Moore 1950 and *Gordona* Tsukamura 1971. Of these, *Rhodococcus* has priority. Although this genus was founded to accommodate pink-pigmented cocci, the only authentic extant strain of the type species, *Rhodococcus rhodochrous*, is undoubtedly a member of the '*rhodochrous*' taxon (see Gordon & Mihm, 1957).

In the present study, carefully chosen gordonae and rhodochrous strains and marker cultures of *Nocardia* and *Mycobacterium* were compared using numerical taxonomic methods in an attempt to clarify the taxonomy of the '*rhodochrous*' complex.

METHODS

Strains. One hundred and forty-two rhodochrous strains, 10 gordonae and 25 'marker' cultures of mycobacteria and nocardiae were examined (Table 1). The 'markers' were 10 strains of rapidly growing mycobacteria belonging to Runyon's group IV (Kubica *et al.*, 1972) and strains of *Nocardia asteroides*, *N. brasiliensis* and *N. caviae*. All of the cultures were maintained on yeast extract agar (YEA) at room temperature (Goodfellow, 1971).

Collection of data. Data for the N-coded rhodochrous strains were taken from earlier studies (Goodfellow, 1971; Goodfellow, Fleming & Sackin, 1972). The remaining strains were examined for purity and screened for 92 unit characters (Table 5).

Table 1. Designation and source of cultures assigned to clusters 1, 2 and 3

(a) Cultures assigned to subcluster 1A	
R5	<i>Bacillus havaniensis</i> ; ATCC4001. 1a*
†R4	<i>Bacillus mycoides corallinus</i> ; ATCC999. 1a*
R6	<i>B. mycoides roseus</i> I; ATCC4004. 1a*
R2	<i>B. mycoides roseus</i> II; ATCC271. 1a*
R10	<i>Bacillus rubricus</i> ; ATCC14341. 1a*
R63	<i>Jensenia</i> sp.; ATCC25721; R. E. Gordon (R.E.G.), Rutgers University, U.S.A., 885. 1a*
†R7	<i>Mycobacterium agreste</i> ; ATCC4273 = 335, 338 (Bradley, 1971) = N22, N61, N84 (Goodfellow, 1971). 1a*
R8	<i>Mycobacterium crystallophagum</i> ; ATCC4276 = 337 (Bradley, 1971). 1a*
R61	<i>Mycobacterium lacticola</i> ; ATCC25717; R.E.G., 584. 14C*
R79	<i>M. lacticola</i> ; ATCC25737; R.E.G.; NCTC8154. 1a*
°N54	<i>Mycobacterium rhodochrous</i> ; ATCC13808; R.E.G., 372; NCIB11147. 14C*. Type strain (<i>Rhodococcus rhodochrous</i>)
N55	<i>M. rhodochrous</i> ; R.E.G., 817. 14C*
N61	<i>M. rhodochrous</i> ; R.E.G., W3408; ATCC4273. 14C*
N67	<i>M. rhodochrous</i> ; NCTC10210. 14C*
N110	<i>M. rhodochrous</i> ; R.E.G., W3639. 14C*
N111	<i>M. rhodochrous</i> ; R.E.G., 562. 14C*
N239	<i>M. rhodochrous</i> ; NCTC8727. 14C*
N240	<i>M. rhodochrous</i> ; NCTC8571. 14C*
R68	<i>M. rhodochrous</i> ; ATCC25726.
R50	<i>Mycobacterium</i> sp.; ATCC25706; R.E.G., 463. 1a*
R12	<i>Nocardia corallina</i> ; ATCC14347. 1a*
R13	<i>N. corallina</i> ; ATCC14348. 1a*
R14	<i>N. corallina</i> ; ATCC14349. 1a*
R15	<i>N. corallina</i> ; ATCC14350. 1a*
†R57	<i>N. corallina</i> ; ATCC25712; R.E.G., 502. 1a*
°R62	<i>N. corallina</i> ; ATCC25719; R.E.G., 624. 1a*
°R70	<i>N. corallina</i> ; ATCC25730; R.E.G., W3406. 1a*
†R74	<i>N. corallina</i> ; ATCC25732; R.E.G., W3408. 1a*
N84	<i>N. corallina</i> ; ATCC4273. 14C*
R73	<i>Nocardia erythropolis</i> ; ATCC25731; R.E.G., W3407 = ATCC4277 = 474 (Bradley, 1971) = N23, N108 (Goodfellow, 1971). 1a*
N73	<i>Nocardia rubra</i> ; laboratory strain. 14C*
N75	<i>N. rubra</i> ; LA1609. 14C*
N83	<i>N. rubra</i> ; S. T. Williams, E40. 14C*
R47	<i>N. rubra</i> ; ATCC25703; R.E.G., 415. 1a*
R75	<i>N. rubra</i> ; ATCC25733; R.E.G., W3639 = N110. 1a*
N5	<i>Nocardia salmonicolor</i> ; NCIB9701. 14C*
R66	<i>Nocardia</i> sp.; ATCC25724; R.E.G., 1082R. 1a*
R67	<i>Nocardia</i> sp.; ATCC25725; R.E.G., 1082S. 1a*
N26	<i>Proactinomyces luteus</i> ; CCM198. 14C*
N27	<i>Proactinomyces phenoli</i> ; NCIB9160. 14C*
N28	<i>Proactinomyces ruber</i> ; CCM269. 14C*
°R9	<i>Rhodococcus rhodochrous</i> ; ATCC13808. 1a*
R27	' <i>rhodochrous</i> '; ATCC25680. 1a*
R32	' <i>rhodochrous</i> '; ATCC25686. 1a*
R38	' <i>rhodochrous</i> '; ATCC25692. 1a*
R41	' <i>rhodochrous</i> '; ATCC25696. 1a*
R42	' <i>rhodochrous</i> '; ATCC25698. 1b*
R45	' <i>rhodochrous</i> '; ATCC25701. 1a*
R64	' <i>rhodochrous</i> '; ATCC25722; R.E.G., 1022. 1a*
(b) Cultures assigned to subcluster 1B	
N30	<i>Mycobacterium rhodochrous</i> ; R.E.G., A12974; ATCC12974. 14B*
N31	<i>M. rhodochrous</i> ; R.E.G., W21. <i>Micrococcus rhodochrous</i> Overbeck. 14B*
(c) Cultures assigned to subcluster 1C	
°N654	<i>Gordona bronchialis</i> ; NCTC10667; M. Tsukamura, Chuba Chest Hospital, Obu, Aichi-ken 474, Japan, 3410. ATCC25592. Type strain
°N660	<i>G. bronchialis</i> ; NCTC10667. Type strain
°N655	<i>Gordona rubra</i> ; NCTC10668; M. Tsukamura, 3605. Type strain
°N657	<i>G. rubra</i> ; NCTC10668; ATCC25593. Type strain
N658	<i>G. rubra</i> ; M. Tsukamura; ATCC60004
†N656	<i>Gordona terrae</i> ; NCTC10669; M. Tsukamura, 3612. Type strain
†N659	<i>G. terrae</i> ; NCTC10669; ATCC25594. Type strain
N662	<i>G. terrae</i> ; H. Mordarska, Hirsfeld Institute, Wrocław, Poland, T6. Type strain
N450	<i>Nocardia corallina</i> ; R. Bönicke, Institut für Experimentelle Biologie und Medizin, Borstel, West Germany, SN5303
N451	<i>N. corallina</i> ; R. Bönicke, SN5302

Table 1 (cont.)

(d) Cultures assigned to subcluster 1D

^g N420	<i>Nocardia pellegrino</i> ; A. Tacquet, Pasteur Institute, Lille, 906
^h N421	<i>N. pellegrino</i> ; A. Tacquet, 906B
ⁱ N422	<i>N. pellegrino</i> ; A. Tacquet, 107
N423	<i>N. pellegrino</i> ; A. Tacquet, 330
N447	<i>N. pellegrino</i> ; R. Bönicke, SN5108
N448	<i>N. pellegrino</i> ; R. Bönicke, SN5104
N449	<i>N. pellegrino</i> ; R. Bönicke, SN5105
N361	<i>Nocardia rubra</i> ; M. Tsukamura, M-1
N362	<i>N. rubra</i> ; M. Tsukamura, M-75; ATCC9356
N363	<i>N. rubra</i> ; M. Tsukamura, M-192; R.E.G., R-680
N453	<i>N. rubra</i> ; R. Bönicke, SN5206
N454	<i>N. rubra</i> ; R. Bönicke, SN5201
N455	<i>N. rubra</i> ; R. Bönicke, SN5203

(e) Cultures assigned to subcluster 1E

N324	<i>Nocardia pellegrino</i> ; ATCC15998. 14A*
^h N325	<i>N. pellegrino</i> ; G. Castelnuovo, Istituto Superiore Di Sanita, Rome, Italy, 906B, 107, 906, 350. 14A*
ⁱ N326	
^g N327	
N328	

(f) Cultures assigned to subcluster 1F

R39	<i>Corynebacterium equi</i> ; ATCC25694. 1c*
R71	<i>C. equi</i> ; ATCC25729; R.E.G.; NCTC1621. 1c*
R76	<i>C. equi</i> ; ATCC25734; R.E.G., N4219; NCTC4219. 1c*
R59	<i>Nocardia restrictus</i> ; ATCC25715; R.E.G., 545. 1c*
R70	<i>N. restrictus</i> ; ATCC25728; R.E.G., 1256. 1c*
R60	<i>Proactinomyces restrictus</i> ; ATCC25716; R.E.G., 566. 1c*

(g) Cultures assigned to subcluster 1G

N146	<i>Corynebacterium fascians</i> ; M. Turner, Nottingham University, 39. 14E*
R69	<i>Corynebacterium rubrum</i> ; ATCC25727; R.E.G., 1240. 1b*
R49	<i>Mycobacterium eos</i> ; ATCC25705; R.E.G., 462. 1b*
N59	<i>Mycobacterium rhodochrous</i> ; R.E.G., 1240. 14D*
N66	<i>M. rhodochrous</i> ; R.E.G., 8139. 14E*
R65	<i>M. rhodochrous</i> ; ATCC25723; R.E.G., 1054. 1b*
R46	<i>Mycobacterium rubrum</i> ; ATCC25702; R.E.G., 384. 1b*
R78	<i>Mycobacterium</i> sp.; ATCC25736; R.E.G.; NCTC8139. 1b*
R58	<i>Nocardia globerula</i> ; ATCC25714; R.E.G., 544. 1b*
N4	<i>Nocardia rubropertincta</i> ; NCIB9664; ATCC14352. 14D*
N113	<i>N. rubropertincta</i> ; V. B. D. Skerman, Queensland University, 134. 14D*
R55	<i>Proactinomyces globerula</i> ; ATCC25710; R.E.G., 494. 1b*
R56	<i>P. globerula</i> ; ATCC25711; R.E.G., 495. 1b*
R17	' <i>rhodochrous</i> '; ATCC25669. 1b*
R18	' <i>rhodochrous</i> '; ATCC25670. 1b*
R19	' <i>rhodochrous</i> '; ATCC25671. 1b*
R20	' <i>rhodochrous</i> '; ATCC25672. 1b*
R21	' <i>rhodochrous</i> '; ATCC25673. 1b*
R22	' <i>rhodochrous</i> '; ATCC25674. 1b*
R23	' <i>rhodochrous</i> '; ATCC25675. 1b*
R24	' <i>rhodochrous</i> '; ATCC25676. 1b*
R25	' <i>rhodochrous</i> '; ATCC25678. 1b*
R26	' <i>rhodochrous</i> '; ATCC25679. 1b*
R28	' <i>rhodochrous</i> '; ATCC25681. 1b*
R29	' <i>rhodochrous</i> '; ATCC25682. 1b*
R30	' <i>rhodochrous</i> '; ATCC25683. 1b*
R31	' <i>rhodochrous</i> '; ATCC25684. 1b*
R33	' <i>rhodochrous</i> '; ATCC25687. 1b*
R34	' <i>rhodochrous</i> '; ATCC25688. 1b*
R35	' <i>rhodochrous</i> '; ATCC25689. 1b*
R43	' <i>rhodochrous</i> '; ATCC25699. 1b*
R51	' <i>rhodochrous</i> '; ATCC25685. 1b*

(h) Cultures assigned to subcluster 1H

N53	<i>Jensenia canicruria</i> ; NCIB8147. 14D*
N143	<i>J. canicruria</i> ; M. Turner, 29. 14D*
N56	<i>Mycobacterium rhodochrous</i> , R.E.G., 1256. 14D*
N57	<i>M. rhodochrous</i> ; R.E.G., 1293S. 14D*
N58	<i>M. rhodochrous</i> ; R.E.G., 1257. 14D*
N60	<i>M. rhodochrous</i> ; R.E.G., 1293R. 14D*

Table 1 (cont.)

N62	<i>M. rhodochrous</i> ; R.E.G., 768. 14D*
N63	<i>M. rhodochrous</i> ; R.E.G., 463. 14D*
N64	<i>M. rhodochrous</i> ; R.E.G., 417; ATCC9356. 14D*
N65	<i>M. rhodochrous</i> ; R.E.G., A7698; ATCC7698. 14D*
N108	<i>M. rhodochrous</i> ; R.E.G., A4277; ATCC4277. 14D*
N109	<i>M. rhodochrous</i> ; R.E.G., 494. 14D*
N148	<i>Nocardia corallina</i> ; M. Turner, 83. 14D*
N11	<i>Nocardia erythropolis</i> ; NCIB9158; ATCC4277. Type strain. 14D*
N23	<i>N. erythropolis</i> ; CCM277; ATCC4277. 14D*
N209	<i>N. erythropolis</i> ; J. E. Thiemann, Lepetit S.p.A., Milan, Italy, A2. 14D*
N12	<i>Nocardia globerula</i> ; NCIB9159; ATCC19370. Type strain. 14D*
N38	<i>Nocardia opaca</i> ; NCIB9409. 14D*
N25	<i>Proactinomyces globerulus</i> ; CCM279. 14D*

(i) Cultures assigned to subcluster 1I

N443	<i>Nocardia rhodnii</i> ; P. Hill, Edinburgh University, A1
N444	<i>N. rhodnii</i> ; P. Hill, A/0
N445	<i>N. rhodnii</i> ; P. Hill, B/0; NCIB11279
N446	<i>N. rhodnii</i> ; P. Hill, B/1

(j) Cultures assigned to subcluster 1J

N650	<i>Rhodococcus coprophilus</i> ; T. J. Rowbotham, Bradford University, CUB628
N651	<i>R. coprophilus</i> ; T. J. Rowbotham, CUB118
N652	<i>R. coprophilus</i> ; T. J. Rowbotham, N38
N653	<i>R. coprophilus</i> ; T. J. Rowbotham, N19

(k) Cultures assigned to cluster 1

N663	<i>Gordona aurantiaca</i> ; NCTC10741. Type strain
N661	<i>Gordona bronchialis</i> ; H. Mordarska, T2; M. Tsukamura, 3411
N10	<i>Nocardia aurantia</i> ; NCIB9557; ATCC12674. 14E*
N452	<i>Nocardia corallina</i> ; R. Bönicke, SN5301
N647	<i>Nocardia kirovani</i> ; G. Michel, Lyons University, IM1374
N124	<i>Nocardia opaca</i> ; CBS330.61. 14E*
N125	<i>Nocardia opaca</i> ; CBS331.61. 14E*
N7	<i>Nocardia restrictus</i> ; NCIB10027. 14E*

(l) 'Markers' assigned to cluster 2

M12	<i>Mycobacterium aurum</i> ; ATCC23366. Type strain
M158	<i>Mycobacterium diernhoferi</i> ; ATCC19340. Type strain
M3	<i>Mycobacterium flavescens</i> ; ATCC14474. Type strain
M93	<i>Mycobacterium fortuitum</i> ; ATCC6841. Type strain
M17	<i>Mycobacterium parafortuitum</i> ; ATCC19686. Type strain
M6	<i>Mycobacterium peregrinum</i> ; ATCC23023. Type strain
M98	<i>Mycobacterium phlei</i> ; ATCC11758. Type strain
M101	<i>Mycobacterium smegmatis</i> ; ATCC11468. Type strain
M11	<i>Mycobacterium thermoresistibile</i> ; ATCC19527. Type strain
M37	<i>Mycobacterium vaccae</i> ; ATCC15483. Type strain

(m) 'Markers' assigned to cluster 3

N13	<i>Nocardia asteroides</i> ; NCTC8595; ATCC14759
N100	<i>N. asteroides</i> ; R.E.G., 652
N105	<i>N. asteroides</i> ; R.E.G., 9969; ATCC9969
N233	<i>N. asteroides</i> ; R. Olds, Cambridge University, CN750
N317	<i>N. asteroides</i> ; ATCC19247. Type strain
N14	<i>Nocardia brasiliensis</i> ; NCTC10300; ATCC19295
N48	<i>N. brasiliensis</i> ; R.E.G., 744. ATCC91295
N311	<i>N. brasiliensis</i> ; F. Mariat, Institut Pasteur, Paris, 768
N312	<i>N. brasiliensis</i> ; F. Mariat, 337
N318	<i>N. brasiliensis</i> ; ATCC19296. Type strain
N36	<i>Nocardia caviae</i> ; NCTC1934; ATCC14629. Type strain
N231	<i>N. caviae</i> ; R. Olds, CN749
N313	<i>N. caviae</i> ; F. Mariat, 751
N562	<i>N. caviae</i> ; D. Frey, Royal North Shore Hospital, The Institute of Medical Research, N.S.W., Australia, 1911
N563	<i>N. caviae</i> ; D. Frey, 1912

* Phena recovered by Goodfellow (1971) or by Goodfellow *et al.* (1974).

† Superior letters preceding laboratory numbers indicate triplicate (a, b) or duplicate (c to i) cultures.

M, N, R, laboratory numbers. ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A. CBS, Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands. CCM, Czechoslovak Collection of Micro-organisms, Brno, Czechoslovakia. LA, Institut d'Hygiene, Lausanne, Switzerland. NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland. NCTC, National Collection of Type Cultures, London.

Seven-day-old YEA cultures were used to inoculate the test media and tests were read after 7 and 14 days incubation at 30 °C. The media and methods have been described previously (Goodfellow, 1971; Lacey & Goodfellow, 1975). Inhibition and nutritional tests were performed in divided dishes (Sneath & Stevens, 1967), degradation studies in Petri dishes and physiological tests in tubes. Cultures grown on modified Sauton's medium were analysed for the presence of lipids characteristic for *Nocardia* (lipid LCN-A) (Mordarska, Mordarski & Goodfellow, 1972). These lipids have been found to be free, short-chain mycolic acids (Goodfellow *et al.*, 1973; Minnikin, Patel & Goodfellow, 1974; Alshamaony *et al.*, 1976*a, b*).

Coding of data. Nearly all of the characters existed in one of two mutually exclusive states and were scored plus (+) or minus (−). 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 allantoinase and urease production, were scored by the additive method of Sokal & Sneath (1963). The binary data were entered on standard IBM punched cards.

Computer analyses. Data were analysed using the matching coefficient S_{SM} (Sokal & Michener, 1958) and sorted similarity matrices were obtained using the unweighted average linkage cluster analysis technique (Sneath & Sokal, 1974). The mean intragroup and intergroup similarity values of the defined clusters were printed after clustering. Vigour and pattern statistics were also calculated (Sneath, 1968).

RESULTS

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

The test strains were recovered in three clusters defined at the 75 to 80 % similarity level (S level) (Fig. 1). One hundred and fifty-two strains provisionally assigned to the genus *Gordona* or the 'rhodochrous' complex grouped into cluster 1; the mycobacteria and nocardiae grouped into clusters 2 and 3 respectively (Fig. 1; Table 1). Most of the cultures in cluster 1 fell into one of 10 subclusters: 1A to 1J (Fig. 1; Table 1). The mean intragroup similarity of each cluster and subcluster and the corresponding intergroup similarities are shown in Tables 2 and 3. The three clusters are well separated and mean intragroup similarities are high.

Cluster 1

Subcluster 1A contains 49 strains (Table 1*a*) including the earliest described rhodochrous strain, *Rhodococcus rhodochrous* (N54 and R9).

Subcluster 1B contains two strains (Table 1*b*) which share a high similarity to those in subcluster 1A (Fig. 1).

Subcluster 1C contains 8 *gordonae* and two strains received as *Nocardia corallina* (Table 1*c*). This taxon exhibits some structure; *Gordona bronchialis* strains (N654, N660) and two of the *G. terrae* strains (N656, N662) can be distinguished from each other and from the remaining strains in the subcluster (Fig. 1). Two *Gordona* strains did not group in the subcluster; *G. aurantiaca* (N663) is only marginally included in cluster 1 but *G. bronchialis* (N661) shows a high affinity to both subclusters 1A and 1C.

Subclusters 1D and 1E share a high intergroup similarity ($\geq 85\%$) and contain strains received as *Nocardia pellegrino*; the former also includes six strains labelled *N. rubra* (Table 1*d, e*).

Fig. 1. Matching matrix of the 177 test cultures based on 92 unit characters.

Table 4. *Distribution of 17 characters with the greatest resolving power for the three clusters*

Results indicate the percentage frequency of positive characters.

Character	Cluster 1 *(152)	Cluster 2 (10)	Cluster 3 (15)
Staining and colony morphology:			
Strongly acid-fast	0	100	0
Mycelium formed	100	0	100
Aerial hyphae moderate to abundant	0	0	100
Margin entire	83	100	0
Elevation convex	81	100	0
Colonies pink, red, orange	85	10	67
Diffusible exopigment	0	0	100
Growth on sole carbon source:			
Ethanol	94	20	0
<i>p</i> -Hydroxybenzoic acid	70	0	0
Sodium octanoate	74	40	0
Testosterone	70	0	67
Growth at 10 °C	73	0	13
Growth in the presence of:			
0.3 % (v/v) Phenylethanol	81	0	20
7.0 % (w/v) Sodium chloride	77	0	27
Susceptibility to 10 i.u. penicillin	91	0	34
Allantoinase weak	15	0	100
LCN-A present	100	0	100

* Number of cultures analysed is given in parentheses.

Subcluster 1F is composed of three strains received as *Corynebacterium equi*, and three as *Nocardia restrictus* or *Proactinomyces restrictus* (Table 1f).

Subcluster 1G contains 32 strains carrying a number of different genus and species names (Table 1g).

Subcluster 1H accommodates 19 strains also received under a variety of names (Table 1h).

Subcluster 1I is composed of four strains of *Nocardia rhodnii* (Table 1i).

Subcluster 1J contains four strains received as *Rhodococcus coprophilus* (Table 1j).

The remaining strains classified in cluster 1 are listed in Table 1k.

Clusters 2 and 3

Cluster 2 contains the marker strains of *Mycobacterium* and cluster 3 the nocardiae (Table 1l, m); the latter are distributed into subclusters (a, b and c) representing *Nocardia asteroides*, *N. caviae* and *N. brasiliensis* respectively (Fig. 1).

Reproducibility of results

Since some identical strains (Table 1) were received from different sources it was possible to get an approximate estimate of the experimental test error. Triplicate cultures were examined simultaneously but some of the duplicate cultures were studied on separate occasions in two different laboratories. As the tests, incubation conditions and scoring procedures were standardized, both intra-laboratory and inter-laboratory errors could be estimated. With cultures examined at the same time, the probability (*p*) of an erroneous

result averaged 3.7 % which is equal to an observed S_{SM} value of around 92 % between duplicate cultures. With cultures studied on two different occasions, the p value was 8.1 %.

Clustering of strains using vigour and pattern statistics

The percentage dissimilarity (D_T) between strains can be divided into vigour (D_V) and pattern (D_P) components (Sneath, 1968). The former expresses the difference between strains in total metabolic activity, as measured by the number of positive reactions. The pattern difference reflects which particular tests give positive results and tries to make allowances for differences in growth rates, periods of incubation and similar factors which would otherwise be included in the overall dissimilarity.

The removal of the vigour component from total dissimilarities did not have any effect on the distribution of strains to the three main clusters and only marginally influenced the composition of the rhodochrous subclusters. Five strains (R35, R55, R56, R58, N59) originally classified in subcluster 1G were regrouped in subcluster 1A and one strain (R74) from the latter was reclassified in subcluster 1G. Three strains (R30, N66, N146) recovered in subcluster 1G were allocated to subcluster 1H.

Characters useful for separation of the 'rhodochrous' taxon from Mycobacterium and Nocardia

Seventeen characters were chosen as having the greatest resolving power for the separation of cultures assigned to the three clusters (Table 4).

Properties and identification of the rhodochrous subclusters

In the phenotypic characterization of the rhodochrous strains several characters were found to have a discriminating value. This was apparent when the frequency of each test which was positive in each of the subclusters had been calculated (Table 5). Fourteen characters were chosen as having the greatest resolving power for the differentiation of the rhodochrous subclusters (Table 6). Most of the subclusters could be distinguished by six or more characters; only subclusters 1D and 1E were difficult to separate.

DISCUSSION

The most stable classifications are those in which the relationships between taxa are consistent when they are based on different kinds of taxonomic criteria, and the degree of confidence that can be placed in a classification is a reflection of the congruence found between the different types of information. Numerical taxonomy and DNA:DNA re-association are two of the more powerful tools in modern taxonomy; however, at present it is not possible to formally equate homology with similarity values or to objectively relate either of them to taxonomic rank. Nevertheless, there seems to be good agreement between nucleotide sequence homology and numerical phenetic data in a number of genera (Sneath, 1972; Colwell *et al.*, 1974; Staley & Colwell, 1973) and in some actinomycete taxa (Gross & Wayne, 1970; Mordarski *et al.*, 1976).

In phenetic taxonomy there is always a danger that results are influenced by differences in growth rates and cultural conditions (Sneath, 1968) and by test errors and the lack of reproducibility of tests; numerical taxonomy has highlighted this (Sneath, 1972, 1976; Sneath & Johnson, 1972). The phenetic data in the present study were based on pattern differences because removal of the vigour component only marginally affected the clustering of the test strains. Test errors between laboratories tend to be greater than those within laboratories

Table 5. *Percentage frequencies of positive characters found in the subclusters of cluster 1*

Character	Subcluster									
	1A *(49)	1B (2)	1C (10)	1D (13)	1E (5)	1F (6)	1G (32)	1H (19)	1I (4)	1J (4)
Colony morphology:										
Aerial hyphae sparse	16	0	0	46	100	0	3	48	0	25
Margin entire	98	100	40	23	0	100	100	100	100	0
Margin filamentous	2	0	0	78	100	0	0	0	0	100
Elevation convex	82	100	60	100	100	0	90	85	100	100
Elevation irregular	18	0	0	0	0	0	9	11	0	0
Colonies white	2	0	0	8	0	17	9	64	25	0
Colonies pink, red, orange	98	100	100	92	100	83	90	32	75	100
Degradation of:										
Adenine	64	100	0	15	100	100	0	95	0	0
Allantoin, weak	2	0	0	0	0	0	6	69	0	0
Tyrosine	96	50	0	85	100	0	9	69	100	0
Urea, weak	56	100	100	85	100	100	96	85	100	100
Urea, strong	56	50	10	0	0	83	78	85	50	0
Growth on sole carbon source (1 %, w/v):										
Glycerol	100	100	100	85	100	100	96	100	50	0
Inositol	0	0	20	0	0	0	3	42	0	0
Inulin	100	100	100	100	100	67	100	100	50	100
Maltose	100	100	10	0	100	0	100	100	0	0
Mannitol	100	100	100	100	100	0	59	100	100	0
Rhamnose	0	0	20	0	0	0	0	0	0	0
Salicin	22	50	0	0	0	17	0	80	0	0
Sorbitol	100	100	80	92	100	0	59	95	100	0
Trehalose	96	100	100	69	100	0	90	100	50	0
Growth on sole carbon source (0.1 %, w/v):										
Acetamide	72	100	60	78	100	33	68	90	100	0
Benzamide	22	0	20	8	100	0	0	0	25	0
<i>p</i> -Cresol	96	100	100	92	100	0	90	21	50	50
<i>m</i> -Hydroxybenzoic acid	80	100	0	100	100	17	6	0	0	100
<i>p</i> -Hydroxybenzoic acid	86	100	70	100	100	83	56	80	50	50
Pimelic acid	68	50	100	100	100	0	12	64	75	100
Sebacic acid	88	100	100	85	100	0	90	100	50	25
Sodium adipate	100	50	100	100	100	33	90	85	100	100
Sodium benzoate	96	50	100	100	100	67	37	5	100	100
Sodium butyrate	100	100	100	100	100	33	100	90	100	100
Sodium citrate	96	100	70	92	100	50	84	85	25	25
Sodium fumarate	96	100	100	100	100	100	81	100	75	75
Sodium gluconate	46	100	80	46	0	0	28	100	100	25
Sodium lactate	92	100	30	23	100	67	22	90	25	0
Sodium malate	100	100	100	100	100	100	87	100	100	0
Sodium octanoate	92	0	100	92	100	83	68	37	100	100
Sodium pyruvate	100	100	30	54	100	100	93	100	0	100
Sodium succinate	98	100	100	100	100	100	93	100	100	0
Sodium tartrate	0	0	20	23	100	0	0	0	0	0
Testosterone	90	0	90	92	100	50	10	95	0	100
L-Tyrosine	88	50	20	92	100	50	90	5	100	0
Growth on sole carbon/nitrogen source:										
Acetamide	56	0	40	69	0	50	53	53	100	0
Growth at:										
10 °C	96	100	0	15	100	100	87	85	0	25
40 °C	100	100	100	100	100	100	96	90	0	75
45 °C	26	0	0	0	0	0	3	5	0	0

Table 5 (cont.)

	Subcluster									
	1A	1B	1C	1D	1E	1F	1G	1H	1I	1J
Growth in the presence of:										
0.001 % (w/v) Crystal violet	96	50	100	100	100	100	93	42	100	100
0.0001 % (w/v) Crystal violet	62	0	100	85	100	100	68	5	50	100
0.1 % (w/v) Phenol	96	100	100	92	100	33	68	42	25	100
0.3 % (v/v) Phenylethanol	98	0	90	100	100	67	81	58	0	75
0.01 % (w/v) Sodium azide	56	0	100	85	100	100	81	80	100	75
0.02 % (w/v) Sodium azide	18	0	90	100	100	50	16	64	25	25
5.0 % (w/v) Sodium chloride	98	0	100	100	100	100	96	95	100	100
7.0 % (w/v) Sodium chloride	76	0	100	92	100	50	84	74	100	100
Susceptibility to 10 i.u. penicillin	92	100	40	100	100	50	96	100	100	100
Enzymic activity:										
Nitrate reduction	100	100	80	100	100	100	96	90	100	100
<i>m</i> -Nitrophenoloxidase	82	50	0	23	0	0	0	5	0	0
<i>p</i> -Nitrophenoloxidase	4	0	10	15	100	17	0	0	0	0

* Number of cultures analysed is given in parentheses.

No strains of subclusters 1A to 1J utilized adonitol, arabinose, cellobiose, galactose, lactose, melezitose, raffinose or xylose as sole carbon sources; nor trimethylenediamine or serine as sole carbon and nitrogen sources. None degraded allantoin (strong), casein, elastin, hypoxanthine, Tween 20, Tween 40, Tween 60. None were acid-fast, nor produced diffusible pigment. Nearly all strains formed mycelium. All utilized ethanol, fructose, mannose, sucrose, sodium acetate and sodium propionate as sole carbon sources; and contained LCN-A.

Table 6. Distribution of 14 characters with the greatest resolving power for the 10 subclusters of cluster 1

Results indicate the percentage frequency of positive characters.

Character	Subcluster									
	1A *(49)	1B (2)	1C (10)	1D (13)	1E (5)	1F (6)	1G (32)	1H (19)	1I (4)	1J (4)
Degradation of:										
Adenine	64	100	0	15	100	100	0	95	0	0
Tyrosine	96	50	0	85	100	0	9	69	100	0
Growth on sole carbon source:										
Glycerol (1 %, w/v)	100	100	100	85	100	100	96	100	50	0
Maltose (1 %, w/v)	100	100	10	0	100	0	100	100	0	0
Mannitol (1 %, w/v)	100	100	100	100	100	0	59	100	100	0
Sorbitol (1 %, w/v)	100	100	80	92	100	0	59	95	100	0
Trehalose (1 %, w/v)	96	100	100	69	100	0	90	100	50	0
<i>p</i> -Cresol (0.1 %, w/v)	96	100	100	92	100	0	90	21	50	50
<i>m</i> -Hydroxybenzoic acid (0.1 %, w/v)	80	100	0	100	100	17	6	0	0	100
Sodium pyruvate (0.1 %, w/v)	100	100	30	54	100	100	93	100	0	100
Testosterone (0.1 %, w/v)	90	0	90	92	100	50	10	95	0	100
Growth at 10 °C	96	100	0	15	100	100	87	85	0	25
Growth in the presence of:										
0.0001 % (w/v) Crystal violet	62	0	100	85	100	100	68	5	50	100
0.02 % (w/v) Sodium azide	18	0	90	100	100	50	16	64	25	25

* Number of cultures analysed is given in parentheses.

(Sneath, 1976), a trend which was again apparent in the present investigation where the probabilities of error were 8.1 % and 3.7 % respectively. Experimental error on this scale can be accommodated in numerical taxonomic surveys (Sneath, 1976) though errors over 10 % are likely to degrade taxonomic structure (Sneath & Johnson, 1972).

Our results, in accordance with the conclusions of earlier studies (Cerbón, 1967; Bradley, 1971; Goodfellow, 1971; Tacquet *et al.*, 1971; Kubica *et al.*, 1972; Goodfellow *et al.*, 1973, 1974) show that the 'rhodochrous' complex forms a taxon equivalent in rank to the genera *Mycobacterium* and *Nocardia*. Less attention has been paid to the relationships between rhodochrous strains and coryneform bacteria but it seems that the 'rhodochrous' complex can be distinguished from taxa such as *Arthrobacter*, *Corynebacterium* and *Brevibacterium linens* (Bousfield, 1972; Jones, 1975; Bousfield & Goodfellow, 1976). The recovery of the gordonae in the 'rhodochrous' complex is in agreement with the results of Tsukamura (1974, 1975) and lends support to his view that gordonae and rhodochrous strains be classified in a single taxon. The new data, based on test strains representing rhodochrous phena recovered in earlier studies, make it timely to evaluate the taxonomic status of the 'rhodochrous' complex and of the phena within it.

Subcluster 1A is substantially the same as *Nocardia rubra* (Bradley, 1971), phenon 14C (Goodfellow, 1971), *Gordona rhodochroa* (Tsukamura, 1971) and phenon 1a (Goodfellow *et al.*, 1974). It contains strains carrying a variety of names, and to those listed in Table 1 can be added *Nocardia blackwellii*, *N. globerula* and *N. opaca* (Bradley, 1971) and *N. salmonicolor*, *Proactinomyces corallinus*, *P. luteus*, *P. phenoli* and *P. ruber* (Goodfellow, 1971). The type strains of *Gordona rosea* (R6) and *Gordona rubropertincta* (Tsukamura, 1973) were recovered in subcluster 1A and phenon 1a (Goodfellow *et al.*, 1974) respectively. The oldest name borne by any strain in subcluster 1A was *Rhodococcus rhodochrous*.

Representatives of subcluster 1A and the equivalent phena form a distinct serological group (Goodfellow *et al.*, 1974; Ridell, 1974), contain free mycolic acids with 38 to 48 carbon units (Alshamaony *et al.*, 1976a), have menaquinones with eight isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977), and form a DNA homology group which can be distinguished from similar groups of rhodochrous strains (Clark *et al.*, 1971; Mordarski *et al.*, 1976, 1977). They have a DNA base composition within the range 62 to 69 mol % GC (Mordarski *et al.*, 1976, 1977). Subcluster 1B, phenon 14B (Goodfellow, 1971), contains only two strains (Table 1b) which should be classified in subcluster 1A. Tsukamura (1973) classified rhodochrous strain N31 as *Gordona rhodochroa* and Mordarski *et al.* (1977) found that subclusters 1A and 1B formed a single DNA homology group. Subcluster 1B strains share a high similarity to subcluster 1A strains (Fig. 1) and failure to recover them in the same subcluster can be attributed to small test and sampling errors. Subcluster 1A contains two strains which originally carried the epithet *rhodochrous*: ATCC13808 (N54) (*R. rhodochrous* Zopf) and N31 (*Micrococcus rhodochrous* Overbeck); the former is the type strain of *Mycobacterium rhodochrous* (Gordon, 1966).

The gordonae in subcluster 1C can be divided into three groups which contain the type strains of *Gordona bronchialis*, *G. rubra* and *G. terrae* respectively (Fig. 1). These taxa have a similar but narrower DNA base composition range than subcluster 1A strains and form DNA homology groups that can be distinguished from each other and from other rhodochrous homology groups (Mordarski *et al.*, 1976, 1977). Gordonae can be separated from most other rhodochrous strains by their antibiotic sensitivity pattern (Goodfellow & Orchard, 1974), their possession of mycolic acids with 52 to 66 carbon units (Alshamaony *et al.*, 1976b) and their possession of menaquinones with nine isoprene units and one hydro-

genated double bond as the prevalent type (Collins *et al.*, 1977). *Nocardia kirovani* (N647) contains free mycolic acids with between 59 and 65 carbon units (Alshamaony *et al.*, 1976*b*). The relationship between *N. kirovani*, *Gordona aurantiaca* and rhodochrous strains needs to be examined in more detail.

Nocardia pellegrino strains form a good taxon on the basis of biochemical (Juhász & Bönicke, 1965; Pietkiewicz, Andrzejewski & Mordarska, 1973), chemical (Mordarska *et al.*, 1973) and phage susceptibility studies (Pietkiewicz *et al.*, 1974). Representative strains form a distinct DNA homology group and have a DNA base composition range of 65 to 69 mol % GC (Mordarski *et al.*, 1976), can be recognized serologically (Ridell, 1974), and contain menaquinones with eight isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977). Strains received as *N. pellegrino* were originally classified as phenon 14A (Goodfellow, 1971), and this taxon was stable when duplicate strains from different sources were added to it (Goodfellow *et al.*, 1972). In the present study, based on fewer unit characters, the same duplicate strains were recovered in subclusters 1D and 1E respectively, a result evidently due to sampling error. The two subclusters share a high intergroup similarity (Table 3), cannot readily be distinguished (Table 5) and subsequently are treated as one taxon. Subcluster 1D contains six strains received as *Nocardia rubra* (Tsukamura, 1971), one of which (N361) was recently described as the type strain of *Gordona lentifragmenta* (Tsukamura *et al.*, 1975).

Subcluster 1F contains strains received as *Corynebacterium equi*, *Nocardia restrictus* and *Proactinomyces restrictus* and is identical to phenon 1c (Goodfellow *et al.*, 1974). Gordon (1966) studied all of these strains, except R39, and classified them with rhodochrous strains which do not produce acid from mannitol or sorbitol. The subcluster contains the type strain of *C. equi* (NCTC1621) which is said to ferment glucose (Davis & Newton, 1969; Rogosa *et al.*, 1974). The type strain does not grow anaerobically and produces acid from glucose oxidatively (Gordon, 1966; D. Jones, personal communication; M. Goodfellow, unpublished data). On thin-layer chromatography *C. equi* strains produce a mycolic acid spot with a low but variable R_f value (Goodfellow, Collins & Minnikin, 1976), and on this basis form a group intermediate between many corynebacteria and rhodochrous strains. They also contain menaquinones with eight isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977).

Subcluster 1G contains a few strains originally classified in phenon 14D and 14E (Goodfellow, 1971) but is substantially the same taxon as phenon 1b (Goodfellow *et al.*, 1974). Representative strains have a DNA base composition of 62 to 67 mol % GC (Mordarski *et al.*, 1977), contain mycolic acids with a similar chain length to those in nocardiae (Alshamaony *et al.*, 1976*a*; D. E. Minnikin, M. Goodfellow & L. Alshamaony, unpublished data) and may contain characteristic precipitinogens (Goodfellow *et al.*, 1974).

Subcluster 1H corresponds to *Nocardia erythropolis* (Bradley, 1971), phenon 14D (Goodfellow, 1971) and cluster F3 (Jones, 1975). The taxon contains strains received under a variety of names (Table 1*h*) to which can be added *Nocardia corallina*, *N. canicruria*, *N. coeliaca*, *N. erythropolis* and *N. opaca* (Bradley, 1971), *Jensenia canicruria*, *Nocardia lutea* and *Proactinomyces canicruria* (Goodfellow, 1971) and *Nocardia calcarea* (NCIB8863) (Jones, 1975). Representative strains form a DNA homology group (Clark *et al.*, 1971; Bradley & Huitron, 1973; Bradley, Brownell & Clark, 1973; Bradley & Mordarski, 1976), have a DNA base composition of 61 to 67 mol % GC (Bradley, 1971; Mordarski *et al.*, 1977), are susceptible to phage ϕ EC (Bradley, 1971), contain mycolic acids with 36 to 48 carbon units (Alshamaony *et al.*, 1976*a*), and have menaquinones with eight isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977).

Subcluster 1I contains strains, labelled *Nocardia rhodnii*, isolated from the intestinal flora of the blood sucking anthropod *Rhodnius prolixus* Ståh. There is a consistent association of strains labelled *N. rhodnii* with *Rhodnius prolixus* and evidence that the relationship is a symbiotic one (Cross *et al.*, 1976); however, the identification of the microsymbiont has not always been based on critical taxonomic criteria. An authentic strain of *N. rhodnii* (Erikson, 1935) produced aerial hyphae and was reduced to a synonym of *Nocardia asteroides* (Gordon & Mihm, 1957); it was recovered in the *Nocardia asteroides* cluster by Goodfellow (1971). Strain N443 has a DNA base composition of 66 mol % GC (Mordarski *et al.*, 1977).

Subcluster 1J contains representatives of *Rhodococcus coprophilus*, a taxon proposed (Rowbotham & Cross, 1976) to accommodate bacteria with the trivial name Lspi (large, spored, pink, irregular) and briefly described by Willoughby (1969). Representative strains have a DNA base composition of 60 to 64 mol % GC (Mordarski *et al.*, 1977).

On the basis of the evidence presented we recommend that the '*rhodochrous*' complex be recognized as a genus in its own right. We agree with those (Tsukamura, 1974; Bousfield & Goodfellow, 1976; Rowbotham & Cross, 1976) who consider that the name *Rhodococcus* Zopf 1891 has priority over *Proactinomyces* (Jensen 1931) Bradley & Bond 1974, *Jensenia* Bisset & Moore 1950 and *Gordona* Tsukamura 1971. An amended description of the genus and of nine of the species within it is given below. A tenth species, *Rhodococcus coprophilus*, has been described by Rowbotham & Cross (1977).

Tsukamura (1974) proposed the names *Rhodococcus aurantiacus*, *R. bronchialis*, *R. rhodochrous*, *R. rosea*, *R. rubropertinctus* and *R. terrae* for the *Gordona* species: *G. aurantiaca* Tsukamura & Mizuno 1971, *G. bronchialis* Tsukamura 1971, *G. rhodochroa* (Zopf 1891) Tsukamura 1973, *G. rosea* (Grotenfelt 1889) Tsukamura 1973, *G. rubropertincta* (Hefferan 1904) Tsukamura 1973 and *G. terrae* Tsukamura 1971 respectively. At present there are insufficient data to recognize *R. rosea* and *R. rubropertinctus* as good taxospecies and they are reduced to synonyms of *R. rhodochrous*. *Rhodococcus coprophilus* forms a good taxospecies. However, further work needs to be done to determine the relationships between '*R. aurantiacus*' and other nocardioform taxa. Of the remaining taxa proposed, one is a new species and the others carry new combinations.

A name has to be found for the taxon containing strains of *Nocardia pellegrino* and *N. rubra*. The epithet *pellegrino* has not been validly published and *N. rubra* (Kruse 1896) Chalmers & Christopherson 1916 was considered (Tsukamura, 1971, 1975) to have priority for a group of organisms carrying the labels *Nocardia convoluta*, *Nocardia erythropolis*, *Nocardia globerula*, *Nocardia lutea*, *Nocardia polychromogenes* and *Nocardia rubropertincta*. This taxon was subsequently transferred to the genus *Gordona* as *G. lentifragmenta* (Tsukamura *et al.*, 1975), the combination *Gordona rubra* having already been used (Tsukamura, 1971). *Gordona rubra* Tsukamura 1971 was subsequently considered to be identical to *Gordona rubropertincta* (Tsukamura, 1973) and the latter was given priority. We propose, therefore, that the taxon containing strains received as *Nocardia pellegrino* and *Nocardia rubra* be called *Rhodococcus rubrus*.

We consider *Gordona rubra* Tsukamura 1971 to be a good species on both phenetic and genetic grounds (Mordarski *et al.*, 1976). In the present study the taxon contained strains received as *Nocardia corallina* and did not cluster with the type strains of *Gordona rubropertincta* Tsukamura 1973. The *Nocardia corallina* strains conformed to Hefferan's delineation of *Bacillus mycoides corallinus* (see Gordon & Mihm, 1957), and the name *Rhodococcus corallinus* is proposed for the taxon.

The combination *Nocardia restrictus* has not been validly published, and *Corynebacterium*

equi Magnusson 1923 has priority over *Proactinomyces restrictus* (Turfitt, 1944). The name *Rhodococcus equi* is proposed for subcluster 1F.

In selecting a name for subcluster 1G the following were considered: *Corynebacterium fascians* (Tilford 1936) Downson 1942, *Corynebacterium rubrum* Crowle 1962, *Mycobacterium rubrum* Söhngen 1913, *Nocardia globerula* (Gray 1928) Waksman & Henrici 1948, *Nocardia rubropertincta* (Hefferan 1904) Waksman & Henrici 1948, and *Proactinomyces globerulus* Reed 1939. *Nocardia rubropertincta* has priority over the other validly published names and the name *Rhodococcus rubropertinctus* is proposed for the taxon.

Subcluster 1H contains strains carrying a number of names, and includes the type strains of *Nocardia erythropolis* (Gray & Thornton 1928) Waksman & Henrici 1948 and *Nocardia globerula* (Gray 1928) Waksman & Henrici 1948. *Nocardia erythropolis* is considered to have priority and the name *Rhodococcus erythropolis* is proposed for the taxon.

The strains classified in subcluster 1I were received as *Nocardia rhodnii*. Although the type strain of *N. rhodnii* (Erikson, 1935) Waksman & Henrici 1948 has been reduced to a synonym of *Nocardia asteroides* (Gordon & Mihm, 1957; Goodfellow, 1971), the name *Rhodococcus rhodnii* is retained for this taxon.

DESCRIPTION OF GENUS AND ENCOMPASSED SPECIES

Emendation of *Rhodococcus* Zopf 1891

(Rho.do.coc'cus. Gr.n. *rhodum* the rose; Gr.n. *coccus* a grain or berry; M.L.neut.n. *Rhodococcus* a red coccus.)

The important characteristics of the genus based on the literature descriptions of the 'rhodochrous' taxon (Gordon & Mihm, 1957; Gordon, 1966; Tsukamura, 1971; Cross & Goodfellow, 1973; Bradley & Bond, 1974; Goodfellow & Orchard, 1974; Lacey & Goodfellow, 1975), and on our own data, are as follows. Rhodococci are aerobic, Gram-positive actinomycetes that are pleomorphic but often form a primary mycelium that soon fragments into irregular elements. When inoculated into a fresh nutrient medium, the rod-shaped and coccoid cells give rise to a primary mycelium. Rhodococci are non-motile, may be partially acid-fast and do not produce endospores or conidia, though some strains show a few feeble aerial hyphae. Colonies may be rough, smooth, mucoid or mycobacterial-like and are usually pigmented buff, pink, orange or red, though colourless variants occur. Most strains grow quickly on standard media at 30 °C but some require vitamin B₁ (thiamin) for growth.

Rhodococci reduce nitrate but do not degrade casein, elastin or hypoxanthine. Acid is formed from fructose, glucose, maltose and mannose, but not from dulcitol, lactose, α -methyl-D-glucoside or raffinose. Ethanol, fructose, glucose, mannose, sucrose, acetate, butyrate, fumarate and propionate are utilized as sole sources of carbon for energy and growth but adonitol, arabinose, cellobiose, erythritol, galactose, lactose, melezitose, raffinose or xylose are not. Most strains are sensitive to lysozyme, benzylpenicillin, cephaloridine, erythromycin, gentamycin, neomycin and tobramycin. They do not exhibit arylsulphatase activity.

The cell wall peptidoglycan contains *meso*-diaminopimelic acid, arabinose and galactose (wall type IV) (Mordarska *et al.*, 1973; Goodfellow *et al.*, 1974; Rowbotham & Cross, 1976). Rhodococci contain mycolic acids and LCN-A (Mordarska *et al.*, 1972; Alshamaony *et al.*, 1976a, b) and the GC content of their DNA ranges from 59 to 69 mol % (T_m , buoyant density) (Mordarski *et al.*, 1976, 1977).

Species have a wide distribution and are found in soil (Tsukamura, 1971), in the intes-

tinal flora of insects, in cow dung, manured meadows and fresh water habitats (Cross & Rowbotham, 1974; Cross *et al.*, 1976), and are often isolated from enrichments with both aliphatic and aromatic hydrocarbons (Fredricks, 1967; Raymond, Jamison & Hudson, 1967).

The type species is *Rhodococcus rhodochrous* (Zopf 1891) Tsukamura 1974.

Because *Rhodococcus* is aerobic, has fragmenting vegetative mycelium, type IV walls and mycolic acids, the genus should be classified in the family *Nocardiaceae* (Lechevalier & Lechevalier, 1970; Prauser, 1970; Cross & Goodfellow, 1973). The species descriptions given below do not include characters described as characteristic of the genus.

Emendation of *Rhodococcus rhodochrous*

(Overbeck 1891; Zopf 1891; Gordon & Mihm 1957) Tsukamura 1974

The species description is based on our own data and that of earlier studies (Goodfellow, 1971; Goodfellow *et al.*, 1974; Tsukamura *et al.*, 1975).

Morphology. On glucose yeast extract agar (GYEA), the primary mycelium soon fragments into rods and cocci. Rough, orange to red colonies are formed on GYEA, Sauton's agar and egg media.

Temperature requirements. Growth at 10 to 40 °C.

Enzymic activity. Allantoinase, nicotinamidase and pyrazinamidase negative. α -Esterase negative; β -esterase weakly positive.

Degradation tests. Tyrosine but not *p*-aminosalicylate (PAS) is degraded.

Fermentation studies. Acid is not produced from adonitol, arabinose, cellobiose, ethanol, galactose, glycogen, inositol, inulin, mannitol, melezitose, rhamnose, sorbitol, sucrose, trehalose or xylose.

Sole carbon requirements. Growth on glycerol, inulin, maltose, mannitol, sorbitol, adipate, benzoate, *p*-cresol, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, malate, octanoate, sebacic acid, succinate, testosterone and pyruvate as sole sources of carbon for energy and growth, but not on glycogen, rhamnose, malonate or tartrate.

Sole carbon and nitrogen sources. Growth on glucosamine and L-glutamate, but not on ethanolamine, serine or trimethylenediamine.

Lipid characters. The organisms contain free mycolic acids with 38 to 48 carbon units (Alshamaony *et al.*, 1976a), have menaquinones with eight isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977), but do not possess mycobactins or nocobactins (Ratledge & Patel, 1976a).

G+C content of DNA. The G+C content is 62 mol % by T_m for the type strain and 62 to 69 % for the other strains tested (Mordarski *et al.*, 1976, 1977).

Type strain. The type strain is N54 (= ATCC13808 = R. E. Gordon 372).

Further comments. The group was described previously as *Nocardia rubra* (Bradley, 1971), phenon 14C (Goodfellow, 1971), *Gordona rhodochroa* (Tsukamura, 1971), and phenon 1a (Goodfellow *et al.*, 1974).

Rhodococcus bronchialis (Tsukamura 1971) emend. Tsukamura 1974

The species description is based on our own data and that of earlier studies (Tsukamura, 1971; Tsukamura *et al.*, 1975).

Morphology. Slightly acid-fast, rod-shaped bacteria which produce rough, pink to red colonies on GYEA, Sauton's agar and egg media.

Temperature requirements. Growth at 28 to 40 °C.

Enzymic activity. Acetamidase, nicotinamidase and pyrazinamidase positive; allantoinase negative. α -Esterase negative; β -esterase and acid phosphatase positive.

Degradation tests. Neither tyrosine nor PAS is degraded.

Fermentation studies. Acid is produced from inositol and trehalose, but not from galactose, rhamnose, sorbitol or xylose.

Sole carbon requirements. Growth on glycerol, inositol, inulin, mannitol, sorbitol, trehalose, adipate, benzoate, *p*-cresol, malate, octanoate, pimelic acid, propan-1-ol, propane-1,2-diol, pyruvate, sebacic acid, succinate and testosterone as sole sources of carbon for energy and growth, but not on arabinose, *m*-hydroxybenzoic acid, malonate or rhamnose.

Sole carbon and nitrogen sources. Growth on acetamide and L-glutamate, but not on benzamide, ethanolamine, serine or trimethylenediamine.

Lipid characters. The organisms contain free mycolic acids with 56 to 66 carbon units (Alshamaony *et al.*, 1976*b*), and have menaquinones with nine isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977).

G + C content of DNA. The G + C content is 65 mol % by T_m for the type strain and 63 % for the other strain tested (Mordarski *et al.*, 1976).

Distribution. Isolated from sputum of patients with pulmonary disease.

Type strain. The type strain is N654 (= ATCC25592 = NCTC10667).

Rhodococcus corallinus (Bergey *et al.* 1923) comb. nov.

The species description is based on our own data and that of Tsukamura (1971).

Morphology. Slightly acid-fast, rod-shaped bacteria which produce rough, orange to red colonies on GYEA, Sauton's agar and egg media.

Temperature requirements. Growth at 28 to 40 °C.

Enzymic activity. Urease and allantoinase positive; acetamidase, nicotinamidase and pyrazinamidase negative.

Degradation tests. Neither tyrosine nor PAS is degraded.

Fermentation studies. Acid is produced from mannitol and sorbitol, but not from arabinose, galactose, inositol, rhamnose or xylose.

Sole carbon requirements. Growth on glycerol, inulin, mannitol, sorbitol, trehalose, adipate, benzoate, butane-2,3-diol, citrate, *p*-cresol, malate, octanoate, pimelic acid, propan-1-ol, sebacic acid, succinate and testosterone as sole sources of carbon for energy and growth, but not on inositol, rhamnose, salicin, *m*-hydroxybenzoic acid, malonate or propane-1,2-diol.

Sole carbon and nitrogen sources. Growth on L-glutamate and ethanolamine, but not on acetamide, benzamide, serine or trimethylenediamine.

Lipid characters. The organisms contain free mycolic acids with 52 to 64 carbon units (Alshamaony *et al.*, 1976*b*).

G + C content of DNA. The G + C content is 67 mol % by T_m for the type strain and for the other strain tested (Mordarski *et al.*, 1976).

Distribution. Soil.

Type strain. The type strain is N657 (= ATCC25593 = NCTC10668).

Rhodococcus erythropolis (Gray & Thornton 1928) comb. nov.

The species description is based on our own data and that of earlier studies (Goodfellow, 1971; Jones, 1975).

Morphology. On GYEA, the primary mycelium soon fragments into rods and cocci. Rough, orange to red colonies are formed on GYEA and Sauton's agar.

Temperature requirements. Growth at 4 to 40 °C.

Enzymic activity. Urease and phosphatase positive.

Degradation tests. Adenine is degraded.

Fermentation studies. Acid is produced from glycerol, sorbitol, sucrose and trehalose, but not from adonitol, arabinose, cellobiose, galactose, glycogen, inulin, melezitose, rhamnose or xylose.

Sole carbon requirements. Growth on glycerol, inulin, maltose, mannitol, sorbitol, trehalose, adipate, gluconate, lactate, malate, pyruvate, sebacic acid, succinate and testosterone as sole sources of carbon for energy and growth, but not on glycogen, inositol, rhamnose, benzamide, *m*-hydroxybenzoic acid, malonate or tartrate.

Sole carbon and nitrogen sources. No growth on serine or trimethylenediamine.

Lipid characters. The organisms contain free mycolic acids with 36 to 48 carbon units (Alshamaony *et al.*, 1976a), have menaquinones with eight isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977) but do not possess mycobactins or nocobactins (Ratledge & Patel, 1976a).

G+C content of DNA. The G+C content is 67 mol % by T_m for the type strain, and 61 to 67 % for the other strains tested (Bradley, 1971; Mordarski *et al.*, 1977).

Distribution. Soil.

Type strain. The type strain is NII (= ATCC4277 = NCIB9158).

Further comments. The group was described previously as *Nocardia erythropolis* (Adams *et al.*, 1970; Bradley, 1971), phenon 14D (Goodfellow, 1971), and cluster F3 (Jones, 1975). *Jensenia canicruria* Bisset & Moore 1950, *Nocardia calcarea* Metcalf & Brown 1957, and *Nocardia globerula* (Gray 1928) Waksman & Henrici 1948 are synonyms of *R. erythropolis*.

Rhodococcus equi (Magnusson 1923) comb. nov.

The species description is based on our own data and that of earlier studies (Gordon, 1966; Goodfellow *et al.*, 1974).

Morphology. Produces filaments on GYEA which soon fragment into rods and coccoid elements. On GYEA, smooth, shiny orange to red colonies with entire margins are formed. Some cultures produce abundant slime which may drip on to the cover of inverted Petri dishes during incubation.

Temperature requirements. Growth at 10 to 40 °C.

Enzymic activity. Urease positive.

Degradation tests. Adenine but not tyrosine is degraded.

Fermentation studies. Acid is not produced from arabinose, inositol, mannitol, rhamnose or sorbitol.

Sole carbon requirements. Growth on glycerol, lactate, malate, pyruvate and succinate as sole sources of carbon for energy and growth but not on inositol, maltose, mannitol, rhamnose, sorbitol, trehalose, butane-2,3-diol, *p*-cresol, mucate, oxalate, pimelic acid, sebacic acid or tartrate.

Sole carbon and nitrogen sources. No growth on serine or trimethylenediamine.

Lipid characters. The organisms contain free mycolic acids (LCN-A), and have menaquinones with eight isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977).

G+C content of DNA. A G+C content of 58.5 mol % has been reported (Hill, 1966).

Distribution. Originally isolated from pneumonia in foals.

Type strain. The type strain is R71 (= ATCC25729 = NCTC1621).

Rhodococcus rhodnii sp. nov.

(rhod'ni.i. M.L.masc.n. *Rhodnius* generic name of the reduvid bug; M.L.gen.n. *rhodnii* of *Rhodnius*.)

The species description is based on our own data.

Morphology. On GYEA, the primary mycelium soon fragments into rods and cocci. Colonies are rough, have entire margins and are usually red.

Temperature requirements. Growth at 25 to 37 °C.

Enzymic activity. Urease positive.

Degradation tests. Tyrosine but not adenine is degraded.

Sole carbon requirements. Growth on mannitol, sorbitol, acetamide, adipate, benzoate, gluconate, malate, octanoate, succinate and L-tyrosine as sole sources of carbon for energy and growth, but not on inositol, maltose, rhamnose, salicin, *m*-hydroxybenzoic acid, pyruvate, tartrate or testosterone.

Sole carbon and nitrogen sources. Growth on acetamide, but not on serine or trimethylenediamine.

Lipid characters. The organisms contain free mycolic acids.

G + C content of DNA. The G + C content of strain N443 is 66 mol % by T_m (Mordarski *et al.*, 1977).

Distribution. Isolated from the reduvid bug *Rhodnius prolixus*.

Type strain. The type strain is N445 (NCIB11279).

Rhodococcus rubrus (Kruse 1896) Tsukamura 1971 comb. nov.

The species description is based on our own data and that of Tsukamura *et al.* (1975).

Morphology. On GYEA, the primary mycelium soon fragments into rods, short rods and cocci. Rough, pink to red colonies are formed on GYEA, Sauton's agar and egg media.

Temperature requirements. Growth at 28 to 40 °C.

Enzymic activity. α - and β -esterase negative. Galactosidase and acid phosphatase negative; acetamidase and urease positive.

Degradation tests. Tyrosine is degraded.

Fermentation studies. Acid is produced from mannitol and sorbitol, but not from inositol, rhamnose or trehalose.

Sole carbon requirements. Growth on glycerol, inulin, mannitol, sorbitol, trehalose, adipate, benzoate, butan-1-ol, 2-methylpropan-1-ol, citrate, *p*-cresol, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, malate, malonate, octanoate, pimelic acid, propan-1-ol, propane-1,2-diol, pyruvate, sebacic acid, succinate, testosterone and L-tyrosine as sole sources of carbon for energy and growth but not on inositol, salicin or rhamnose.

Sole carbon and nitrogen sources. Growth on acetamide, glutamate, glucosamine and ethanolamine, but not on benzamide, serine or trimethylenediamine.

Lipid characters. The organisms contain free mycolic acids with 38 to 50 carbon units (Alshamaony *et al.*, 1976a), have menaquinones with eight isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977), but do not possess mycobactins or nocobactins (Ratledge & Patel, 1976a).

G + C content of DNA. The G + C content is 65 to 69 mol % by T_m for the strains tested (Mordarski *et al.*, 1976, 1977).

Type strain. The type strain is N361 (= M. Tsukamura M-1).

Rhodococcus rubropertinctus (Hefferan 1904) comb. nov.

The species description is based on our own data and that of Goodfellow *et al.* (1974).

Morphology. On GYEA, the primary mycelium soon fragments into rods and cocci. Colonies are pink to red, rough with entire margins.

Temperature requirements. Growth at 10 to 40 °C.

Enzymic activity. Urease positive; allantoinase and benzamidase negative.

Degradation tests. Neither adenine nor tyrosine is degraded.

Sole carbon requirements. Growth on glycerol, inulin, maltose, trehalose, adipate, *p*-cresol, pyruvate, sebacic acid, succinate and L-tyrosine as sole sources of carbon for energy and growth, but not on inositol, rhamnose, salicin, benzamide, *m*-hydroxybenzoic acid, tartrate or testosterone.

Sole carbon and nitrogen sources. No growth on serine or trimethylenediamine.

Lipid characters. The organisms contain free mycolic acid (LCN-A type *asteroides*), but do not possess mycobactins or nocobactins (Ratledge & Patel, 1976*a*).

G + C content of DNA. The G + C content is 62 to 67 mol % by T_m for the strains tested (Mordarski *et al.*, 1976, 1977).

Type strain. The type strain is N4 (= ATCC14352 = NCIB9664).

Further comments. The group was described previously as phenon 1*b* (Goodfellow *et al.*, 1974).

Rhodococcus terrae (Tsukamura 1971) emend. Tsukamura 1974

The species description is based on our own data and that of earlier studies (Tsukamura, 1971; Tsukamura *et al.*, 1975).

Morphology. Slightly acid-fast, rod-shaped bacteria which produce rough, pink to orange colonies on GYEA, Sauton's agar and egg media.

Temperature requirements. Growth at 28 to 40 °C.

Enzymic activity. Urease, allantoinase, nicotinamidase, pyrazinamidase positive; acetamidase and acid phosphatase negative. β -Esterase positive; α -esterase negative.

Degradation tests. Neither tyrosine nor PAS is degraded.

Fermentation studies. Acid is produced from mannitol, rhamnose, sorbitol and trehalose but not from arabinose, galactose, inositol or xylose.

Sole carbon requirements. Growth on glycerol, inulin, mannitol, rhamnose, sorbitol, trehalose, adipate, benzoate, *p*-cresol, malate, octanoate, pimelic acid, sebacic acid and succinate as sole sources of carbon for energy and growth, but not on inositol, butane-2,3-diol, malonate or propane-1,2-diol.

Sole carbon and nitrogen sources. Growth on L-glutamate and ethanolamine, but not on acetamide, benzamide or serine.

Lipid characters. The organisms contain free mycolic acids with 52 to 64 carbon units (Alshamaony *et al.*, 1976*b*) and have menaquinones with nine isoprene units and one hydrogenated double bond as the prevalent type (Collins *et al.*, 1977).

G + C content of DNA. The G + C content is 64 mol % by T_m for the type strain and 69 % for the other strain tested (Mordarski *et al.*, 1976).

Distribution. Soil.

Type strain. The type strain is N659 (= ATCC25594 = NCTC10669).

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