A Comparative Study of the 'rhodochrous' Complex and Related Taxa by Delayed-type Skin Reactions on Guinea Pigs and by Polyacrylamide Gel Electrophoresis

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SUMMARY

Cell extracts prepared by ultrasonic disruption of 17 strains of the 'rhodochrous' complex and related taxa were compared by polyacrylamide gel electrophoresis and, for immunologic relatedness, by skin test reactions. Two organisms, *Jensenia canicruria* and *Nocardia calcarea*, gave similar gel patterns and skin test reactions, and are considered to be identical. Extracts of *Nocardia rubra* showed a strong antigenic relationship with those of three *Nocardia pellegrino* organisms (N325, N324 and N420) previously assigned to the 'rhodochrous' complex. Two Gordona organisms appeared to be less antigenically related to the 'rhodochrous' complex. Extracts of three of four organisms designated Lspi (*Rhodococcus coprophilus* Rowbotham & Cross 1976) elicited skin test reactions similar to those of the 'rhodochrous' strains. One Lspi strain, N650, showed striking similarities to the 'rhodochrous' complex strain N420 (*Nocardia pellegrino*).

INTRODUCTION

Nocardioforms are diverse organisms, varying in their biochemical, morphological and nutritional requirements, but they all form a primary mycelium which fragments into rods and cocci. Many of these bacteria have been classified in the genera *Nocardia*, *Mycobacterium* or in the 'rhodochrous' complex (Cross & Goodfellow, 1973). Recently, methods for phage typing, determination of wall composition and lipid content, and immunological studies have been explored to identify good diagnostic characters. In a numerical taxonomic study, Goodfellow (1971) classified 283 nocardioform bacteria into 15 clusters, one of which was equated with the 'rhodochrous' complex (Goodfellow, 1971; Goodfellow *et al.*, 1974). In the present studies, extracts of 14 selected organisms of the 'rhodochrous' complex and three other species were analysed for relatedness by their reactivities in skin testing on sensitized guinea pigs and by polyacrylamide gel electrophoresis (PAGE).

METHODS

Strains. The test strains were from Dr M. Goodfellow, University of Newcastle upon Tyne (Table 1).

Cultures. Cultures were grown on oleic acid albumin and Lowenstein-Jensen egg yolk slants for 2 to 4 days, then inoculated into Blake bottles containing 200 ml of Long's synthetic medium (Darzins, 1958; Drea & Andrejew, 1953). Most strains were harvested within a

Table 1. *Species tested (clusters are those designated by Goodfellow, 1971)*

Cluster 14 (subgroup 14A)	
N324	' <i>rhodochrous</i> ' strain (<i>Nocardia pellegrino</i>); G. Castelnuova, Istituto Superiore Di Sanita, Rome, Italy; ATCC15998
N325	' <i>rhodochrous</i> ' strain (<i>Nocardia pellegrino</i>); G. Castelnuova, 906B
N420	' <i>rhodochrous</i> ' strain (<i>Nocardia pellegrino</i>); A. Tacquet, University of Lille, France, 906
Cluster 14 (subgroup 14C)	
N5	' <i>rhodochrous</i> ' strain (<i>Nocardia salmonicolor</i>); NCIB9701
N54	' <i>rhodochrous</i> ' strain (<i>Mycobacterium rhodochrous</i>); ATCC13808; R. E. Gordon, Rutgers University, U.S.A., 372; (<i>Rhodococcus rhodochrous</i>)
Cluster 14 (subgroup 14D)	
N38	' <i>rhodochrous</i> ' strain (<i>Nocardia opaca</i>); NCIB9409
N53	' <i>rhodochrous</i> ' strain (<i>Jensenia canicruria</i>); NCIB8147; ATCC11048
Cluster 14 (subgroup 14E)	
N124	' <i>rhodochrous</i> ' strain (<i>Nocardia opaca</i>); CBS330.61
Cluster 1 (subgroup 1A)	
N99	<i>Nocardia asteroides</i> ; R. E. Gordon, 508
Other strains	
N41	' <i>rhodochrous</i> ' strain (<i>Nocardia calcarea</i>); NCIB8863
N361	' <i>rhodochrous</i> ' strain (<i>Nocardia rubra</i>); M. Tsukamura, National Chubu Hospital, Obu, Aichi 474, Japan, M-1
N655	<i>Gordona rubra</i> ; NCTC10668; M. Tsukamura, 3605
N656	<i>Gordona terrae</i> ; NCTC10669; M. Tsukamura, 3623
N650 to N653	Lspi strains; T. J. Rowbotham, University of Bradford, CUB628, 118, 670, 669

few days, but N656, N54 (*Mycobacterium rhodochrous*) and the Lspi strains, N650 to N653, required 1 to 2 weeks incubation. Although Lspi strains are reported to require thiamin for growth (Rowbotham & Cross, 1976), these strains, as well as N54, grew abundantly when the inoculum size and incubation period were increased. The same medium for all cultures was preferred in order to minimize variables.

Preparation of antigens. Organisms were washed three times with sterile distilled water by centrifugation at 2000 *g* at 4 °C for 15 min. A portion of the packed organisms was dried on a watch glass at 35 °C and used for sensitizing guinea pigs; the remainder was disrupted ultrasonically and used for skin testing and for polyacrylamide gel electrophoresis. Disruption was achieved by treating a 50% (w/v) suspension of packed organisms in sterile distilled water for 5 to 10 min at maximum output in a Bronson W-350 cell disruptor cooled with circulating ice water. The extracts were then centrifuged for 20 to 30 min at 26000 *g* at 4 °C. The supernatant fluid was sterilized by passage through a 0.2 µm Nalgene filter and stored in 3 ml portions at -20 °C.

Sensitization and skin testing of guinea pigs. Groups of eight Hartley strain female guinea pigs weighing between 300 and 400 g were injected subcutaneously in the nuchal area with 0.2 ml of an emulsion containing 2 mg dried organisms. The emulsions were made with Arlacel A (Atlas Chemicals)/Drakeol 6VR (Penreco)/saline (35:65:100, by vol.). Six weeks after sensitization, the activity of the extracts was screened in four of the sensitized guinea pigs by skin testing with 0.1 ml saline containing various amounts of the homologous antigen. Skin reactions were read after 24 h. A dose was selected which produced an erythematous reaction of approximately 13 mm diam. This dose, determined for each of the homologous systems, will be referred to as the bioequivalent dose.

The eight guinea pigs in each group, including a group of control animals which had not

previously been sensitized, were then skin tested with 0.1 ml of each of the bioequivalent skin test doses. Since the four animals which had been used to determine each bioequivalent skin test dose reacted in the same way as those not previously tested, the results were pooled and analysed according to Magnusson's procedure for specificity differences (Magnusson, Engbaek & Bentzon, 1961; Magnusson, 1971, 1973).

Polyacrylamide gel electrophoresis. Electrophoresis was performed as described by Davis (1964), using the Canaco Disc Electrophoresis apparatus, model 1200 (5635 Fishers Lane, Rockville, Maryland 20852, U.S.A.) with 75 mm long gel tubes at 5 mA per tube for 35 to 40 min. The separating gel contained acrylamide (7%, w/v) at pH 8.9 and the stacking gel contained acrylamide (2.5%, w/v) at pH 6.7 (Davis, 1964). Gel patterns were compared by measuring the relative mobilities of prominent bands.

RESULTS AND DISCUSSION

The mean homologous and heterologous skin test reactions were determined for the extracts of the 17 strains at the bioequivalent dose (Table 2). The results of skin tests for all the control animals were negative. Some heterologous antigens gave an equal or slightly greater reaction than that given by the homologous antigen.

The specificity differences (SPD) between each of the 17 organisms were analysed according to Magnusson's method (Table 3). A small SPD indicates a close relationship between strains and several groups have been indicated (groups I to III).

One striking finding was that rhodochrous strain N324 (*N. pellegrino*) cross-reacted extensively with all rhodochrous and Lspi strains, as indicated by the low SPD values, but not with *N. asteroides* or the Gordona species. Thus, N324 was a good indicator strain for unifying the rhodochrous and Lspi strains, as well as closely related strains. Another interesting result was that type strain N54 (*M. rhodochrous*) had low SPD values with all Lspi strains, including N652.

Gels with similar PAGE patterns have been grouped together in Fig. 1. Each group (I to IV) includes gels with corresponding band numbers, as discussed below.

Strains from subgroup 14D, which includes N53 (*Jensenia canicruria*) and N38 (*Nocardia opaca*), have been linked above the 90% S (similarity) level (Goodfellow, 1971) and were very similar to N41 (*Nocardia calcaerea*). To facilitate comparison of bands in PAGE patterns of these three organisms, the corresponding prominent bands in each gel have been numbered (Fig. 1, group III). Although the rates of migration of the bands varied among some of the preparations, the similarities of the numbered bands were confirmed by measurements of relative mobilities (Table 4). (Some of the labelled bands are not as distinct in the photograph as they were in the actual gel.) Strains N38 (*N. opaca*) and N53 (*J. canicruria*) shared at least 12 bands (3 and 7 to 17), a finding consistent with the reported numerical phenetic relationship (Goodfellow, 1971) and with the low SPD value of 0.54. Strains N41 (*N. calcaerea*) and N53 (*J. canicruria*) also shared a low SPD value of 0.38 (Table 3) and the relative mobilities of all discernible bands were identical (Table 4). The findings, therefore, strongly suggest that *N. calcaerea* should be classified in subgroup 14D (Goodfellow, 1971).

Representative strains from subgroup 14A, which includes rhodochrous strains N324, N325 and N420, have been grouped at the 95% S-level (Goodfellow, 1971). The PAGE patterns (Fig. 1) support this close relationship, and also suggest that strain N361 (*Nocardia rubra*) should be classified in subgroup 14A. There were at least 10 bands (3 to 8, 12, 13, 15 and 17) shared by each of the four organisms (Table 4). An especially close resemblance was seen between N325 and N420 which both displayed the additional bands 1, 2, 9, 14 and

Table 2. *Homologous and heterologous skin test reactions for extracts of 17 rhodochrous and allied strains*

Homologous reactions (shown in bold type) are the same as Aa and Bb reactions in Table 3; heterologous reactions are the same as Ab and Ba reactions in Table 3. The results show the mean diameters (in mm) of eight reactions ± 1 sd. Doses were predetermined for bioequivalence as described in Methods.

Guinea pig sensitizing organism	Challenge antigen															
	N54/ 14C	N650	N653	N361	N325/ 14A	N324/ 14A	N651	N420/ 14A	N5/ 14C	N38/ 14D	N124/ 14E	N652	N41	N53/ 14D	N655	N99
N54/14C (<i>M. rhodochrous</i>)	12.5 $\pm$ 1.9	10.2 $\pm$ 1.7	10.6 $\pm$ 1.1	10.0 $\pm$ 0.9	9.1 $\pm$ 2.1	9.4 $\pm$ 1.6	10.6 $\pm$ 1.6	9.0 $\pm$ 1.7	12.8 $\pm$ 2.0	12.4 $\pm$ 2.5	10.1 $\pm$ 1.6	9.9 $\pm$ 1.2	8.9 $\pm$ 1.2	8.9 $\pm$ 1.0	8.7 $\pm$ 1.7	5.0 $\pm$ 2.7 8.7 $\pm$ 0.8
N650 (<i>Lspi</i>)	14.3 $\pm$ 1.5	13.5 $\pm$ 1.3	13.0 $\pm$ 1.6	12.0 $\pm$ 1.4	12.9 $\pm$ 1.1	12.4 $\pm$ 1.0	11.7 $\pm$ 1.4	12.0 $\pm$ 1.5	13.4 $\pm$ 0.8	14.4 $\pm$ 1.2	13.9 $\pm$ 1.1	11.9 $\pm$ 1.1	12.6 $\pm$ 1.4	9.8 $\pm$ 0.7	9.9 $\pm$ 1.7	7.7 $\pm$ 1.1 10.1 $\pm$ 0.7
N653 (<i>Lspi</i>)	13.4 $\pm$ 1.3	12.1 $\pm$ 0.6	14.4 $\pm$ 1.2	12.4 $\pm$ 0.7	12.5 $\pm$ 0.8	12.3 $\pm$ 0.7	11.6 $\pm$ 1.7	11.0 $\pm$ 1.2	12.4 $\pm$ 1.1	13.0 $\pm$	13.1 $\pm$ 1.5	11.6 $\pm$ 0.9	11.5 $\pm$ 1.5	8.6 $\pm$ 2.0	10.8 $\pm$ 0.7	6.9 $\pm$ 0.6 10.9 $\pm$ 1.0
N361 (<i>N. rubra</i>)	12.6 $\pm$ 0.9	13.0 $\pm$ 1.5	14.5 $\pm$ 1.1	12.0 $\pm$ 1.4	12.8 $\pm$ 1.5	11.4 $\pm$ 1.1	10.4 $\pm$ 1.1	11.3 $\pm$ 1.7	11.8 $\pm$ 1.5	12.3 $\pm$ 2.2	12.1 $\pm$ 1.5	11.7 $\pm$ 1.8	11.3 $\pm$ 1.8	9.8 $\pm$ 2.0	10.1 $\pm$ 2.2	8.1 $\pm$ 1.4 8.6 $\pm$ 1.4
N325/14A (<i>N. pellegrino</i>)	12.1 $\pm$ 1.6	12.0 $\pm$ 0.9	10.9 $\pm$ 1.6	12.4 $\pm$ 1.2	12.5 $\pm$ 1.7	11.4 $\pm$ 1.8	11.0 $\pm$ 1.3	11.8 $\pm$ 1.5	11.5 $\pm$ 2.6	11.5 $\pm$ 2.1	11.9 $\pm$ 1.9	10.3 $\pm$ 1.6	10.4 $\pm$ 2.7	8.1 $\pm$ 3.0	9.2 $\pm$ 1.3	6.5 $\pm$ 1.2 8.2 $\pm$ 1.2
N324/14A (<i>N. pellegrino</i>)	14.4 $\pm$ 1.7	11.0 $\pm$ 1.7	11.8 $\pm$ 1.4	11.9 $\pm$ 1.0	11.4 $\pm$ 0.7	11.5 $\pm$ 1.3	10.6 $\pm$ 1.1	10.6 $\pm$ 1.1	13.3 $\pm$ 2.4	12.9 $\pm$ 1.5	13.3 $\pm$ 1.3	10.3 $\pm$ 0.9	11.6 $\pm$ 1.7	12.3 $\pm$ 1.0	9.3 $\pm$ 1.6	7.0 $\pm$ 1.5 8.5 $\pm$ 1.3
N651 (<i>Lspi</i>)	15.0 $\pm$ 2.9	11.4 $\pm$ 2.5	13.1 $\pm$ 2.1	11.6 $\pm$ 3.1	11.6 $\pm$ 2.3	11.5 $\pm$ 2.5	12.1 $\pm$ 3.7	10.3 $\pm$ 2.8	13.3 $\pm$ 3.8	14.4 $\pm$ 2.5	13.3 $\pm$ 1.9	11.9 $\pm$ 3.1	11.9 $\pm$ 2.6	10.7 $\pm$ 2.6	9.8 $\pm$ 2.8	8.4 $\pm$ 1.6 10.9 $\pm$ 2.5
N420/14A (<i>N. pellegrino</i>)	13.1 $\pm$ 1.7	13.4 $\pm$ 1.3	13.1 $\pm$ 2.8	14.5 $\pm$ 2.2	13.1 $\pm$ 1.6	12.3 $\pm$ 1.8	12.0 $\pm$ 1.5	12.9 $\pm$ 1.0	11.4 $\pm$ 2.6	12.3 $\pm$ 2.3	12.9 $\pm$ 1.7	10.8 $\pm$ 1.4	11.6 $\pm$ 1.8	10.3 $\pm$ 2.5	10.1 $\pm$ 2.3	7.9 $\pm$ 2.2 9.4 $\pm$ 1.6
N51/14C (<i>N. salmonicolor</i>)	10.8 $\pm$ 1.0	10.0 $\pm$ 1.3	10.6 $\pm$ 2.0	10.4 $\pm$ 1.7	10.3 $\pm$ 1.4	9.5 $\pm$ 1.9	10.3 $\pm$ 1.7	8.9 $\pm$ 1.7	12.9 $\pm$ 1.7	10.3 $\pm$ 1.3	10.6 $\pm$ 1.4	9.5 $\pm$ 1.7	9.3 $\pm$ 2.0	9.9 $\pm$ 1.1	8.1 $\pm$ 3.4	6.0 $\pm$ 0.6 8.3 $\pm$ 0.7
N38/14D (<i>N. opaca</i>)	11.0 $\pm$ 1.0	9.7 $\pm$ 1.9	11.4 $\pm$ 2.5	9.3 $\pm$ 2.1	10.0 $\pm$ 1.9	10.0 $\pm$ 1.9	8.1 $\pm$ 2.0	8.3 $\pm$ 2.1	12.7 $\pm$ 2.8	12.1 $\pm$ 2.3	10.0 $\pm$ 1.5	10.4 $\pm$ 2.6	12.3 $\pm$ 1.1	14.1 $\pm$ 2.0	9.4 $\pm$ 2.2	4.4 $\pm$ 1.3 7.6 $\pm$ 2.1
N124/14E (<i>N. opaca</i>)	11.6 $\pm$ 2.3	10.0 $\pm$ 1.3	12.0 $\pm$ 1.7	9.5 $\pm$ 1.1	11.5 $\pm$ 1.6	12.6 $\pm$ 2.3	9.4 $\pm$ 1.2	8.6 $\pm$ 1.2	12.4 $\pm$ 2.7	11.9 $\pm$ 2.0	13.3 $\pm$ 2.1	11.0 $\pm$ 2.0	11.4 $\pm$ 1.6	12.6 $\pm$ 2.0	9.9 $\pm$ 1.5	7.3 $\pm$ 1.0 8.5 $\pm$ 1.4
N652 (<i>Lspi</i>)	14.1 $\pm$ 0.8	10.9 $\pm$ 1.1	13.4 $\pm$ 1.2	9.8 $\pm$ 1.7	11.9 $\pm$ 1.0	11.4 $\pm$ 1.1	10.6 $\pm$ 1.3	8.8 $\pm$ 1.0	12.5 $\pm$ 1.4	13.9 $\pm$ 1.5	13.8 $\pm$ 1.3	13.9 $\pm$ 1.9	13.9 $\pm$ 1.5	9.1 $\pm$ 1.9	11.3 $\pm$ 1.0	7.4 $\pm$ 0.9 10.5 $\pm$ 0.8
N41 (<i>N. calcaraea</i>)	11.5 $\pm$ 1.3	10.3 $\pm$ 2.3	11.0 $\pm$ 2.1	10.3 $\pm$ 1.2	10.8 $\pm$ 1.8	11.0 $\pm$ 1.6	8.5 $\pm$ 1.3	9.0 $\pm$ 1.7	13.6 $\pm$ 1.3	11.4 $\pm$ 1.6	11.3 $\pm$ 0.7	10.0 $\pm$ 2.4	13.9 $\pm$ 1.7	14.4 $\pm$ 3.0	7.9 $\pm$ 1.5	3.3 $\pm$ 2.0 6.4 $\pm$ 2.8
N53/14D (<i>Jensenia canicruria</i>)	12.4 $\pm$ 3.1	10.1 $\pm$ 1.8	11.3 $\pm$ 1.9	10.3 $\pm$ 1.4	10.1 $\pm$ 2.1	10.6 $\pm$ 1.7	9.4 $\pm$ 2.3	8.8 $\pm$ 2.0	10.1 $\pm$ 1.5	10.6 $\pm$ 0.9	11.3 $\pm$ 2.0	11.0 $\pm$ 3.0	12.3 $\pm$ 2.4	13.1 $\pm$ 2.3	9.3 $\pm$ 2.1	6.5 $\pm$ 1.1 7.5 $\pm$ 1.2
N655 (<i>G. rubra</i>)	13.0 $\pm$ 1.9	11.3 $\pm$ 1.8	12.3 $\pm$ 1.5	10.3 $\pm$ 1.9	11.6 $\pm$ 2.4	11.8 $\pm$ 2.1	10.7 $\pm$ 2.4	9.7 $\pm$ 1.8	11.0 $\pm$ 2.2	12.8 $\pm$ 1.2	13.3 $\pm$ 1.0	11.7 $\pm$ 1.8	12.9 $\pm$ 1.6	10.1 $\pm$ 1.6	14.9 $\pm$ 1.3	9.7 $\pm$ 2.0 9.1 $\pm$ 1.1
N656 (<i>G. terrae</i>)	11.8 $\pm$ 1.8	10.9 $\pm$ 1.6	12.0 $\pm$ 1.9	9.9 $\pm$ 2.0	11.3 $\pm$ 1.9	10.6 $\pm$ 2.4	9.4 $\pm$ 1.2	8.3 $\pm$ 1.8	10.4 $\pm$ 1.9	12.8 $\pm$ 2.3	12.3 $\pm$ 1.6	10.3 $\pm$ 2.0	11.6 $\pm$ 2.3	11.3 $\pm$ 2.0	13.4 $\pm$ 0.8	11.8 $\pm$ 1.6 9.4 $\pm$ 1.6
N99 (<i>N. asteroides</i>)	12.6 $\pm$ 0.7	8.9 $\pm$ 1.0	10.9 $\pm$ 1.4	8.9 $\pm$ 2.0	8.9 $\pm$ 1.9	9.6 $\pm$ 1.5	15.6 $\pm$ 1.5	7.4 $\pm$ 1.8	11.4 $\pm$ 2.0	11.4 $\pm$ 0.8	10.6 $\pm$ 1.4	9.3 $\pm$ 1.8	8.4 $\pm$ 1.5	9.4 $\pm$ 2.0	6.8 $\pm$ 1.9	5.9 $\pm$ 1.2 13.5 $\pm$ 1.8

Specificity difference (SPD) is defined as $(Aa + Bb) - (Ab + Ba)$, in which Aa and Bb are the diameters of the homologous A and B reactions respectively, and Ab and Ba are the diameters of the heterologous A and B reactions respectively. A and B indicate guinea pigs sensitized with strain A and strain B emulsions respectively. For details, see Magnusson *et al.* (1961) and Magnusson (1971, 1973).

	N54/14C (<i>M. rhodochrous</i>)	N650 (<i>Lspi</i>)	N653 (<i>Lspi</i>)	N361 (<i>N. rubra</i>)	N325/14A (<i>N. pellegrino</i>)	N324/14A (<i>N. pellegrino</i>)	N651 (<i>Lspi</i>)	N420/14A (<i>N. pellegrino</i>)	N5/14C (<i>N. salmonicolor</i>)	N38/14D (<i>N. opaca</i>)	N124/14E (<i>N. opaca</i>)	N652 (<i>Lspi</i>)	N41 (<i>N. calcarea</i>)	N53/14D (<i>J. canicruria</i>)	N655 (<i>G. rubra</i>)	N656 (<i>G. terrae</i>)	N99 (<i>N. asteroides</i>)
N54/14C (<i>M. rhodochrous</i>)	–																
N650 (<i>Lspi</i>)	1.4	–															
N653 (<i>Lspi</i>)	2.9	2.7	–														
N361 (<i>N. rubra</i>)	1.9	2.1	–0.5	–													
N325/14A (<i>N. pellegrino</i>)	3.8	1.1	3.5	–0.6	–												
N324/14A (<i>N. pellegrino</i>)	0.3	1.5	1.9	0.3	1.2	–											
N651 (<i>Lspi</i>)	–1.0	2.4	1.8	4.5	2.1	1.5	–										
N420/14A (<i>N. pellegrino</i>)	3.3	0.93	3.1	–0.88	0.5	1.5	7.3	–									
N5/14C (<i>N. salmonicolor</i>)	–0.13	2.9	4.3	2.8	1.6	1.5	1.5	5.5	–								
N38/14D (<i>N. opaca</i>)	1.3	1.4	–2.3	2.6	2.1	0.8	1.8	4.5	2.1	–							
N124/14E (<i>N. opaca</i>)	4.0	2.8	2.5	3.6	2.4	–1.1	2.8	4.6	3.1	3.5	–						
N652 (<i>Lspi</i>)	2.4	4.6	3.3	4.5	4.3	3.0	5.5	7.3	4.8	3.5	2.3	–					
N41 (<i>N. calcarea</i>)	5.0	4.5	5.8	4.4	5.3	2.8	5.6	6.1	3.9	2.4	4.5	6.9	–				
N53/14D (<i>J. canicruria</i>)	4.4	6.6	7.6	5.1	7.4	1.8	5.2	7.0	6.0	0.54	1.8	6.9	0.38	–			
N655 (<i>G. rubra</i>)	5.6	7.1	6.7	6.5	6.6	5.4	6.5	7.9	8.6	4.7	5.0	5.8	8.0	8.6	–		
N656 (<i>G. terrae</i>)	6.5	1.7	7.3	5.8	6.5	5.6	6.1	8.5	9.3	6.7	5.5	8.0	6.7	7.1	3.5	–	
N99 (<i>N. asteroides</i>)	4.7	7.9	6.1	8.0	8.8	8.9	–0.88	9.6	9.8	7.7	7.6	7.6	12.6	9.8	12.5	10.0	–

Subgroup 14C included strains N5 (*Nocardia salmonicolor*) and N54 (*M. rhodochrous*), which have been linked above the 90% S-level (Goodfellow, 1971). The SPD value of 0.13 supports a close relationship between the two organisms, although the affinity was not as obvious in the disc gel patterns (Fig. 1, group II). The extract of strain N54 gave more bands than that of strain N5, but at least eight corresponding bands were discerned.

Three of the four Lspi strains, N650, N651 and N653, shared relatively low SPD values (1.8 to 2.7). Strains N650 and N653 shared the most bands (Table 4). Strains N651 and N653 shared the lowest SPD value of 1.8, and the R_F values of five of their bands were similar (Table 4). The fourth Lspi strain, N652, shared a somewhat higher SPD value (3.3 to 4.6) with each of the other three strains. Strain N652 had three bands with similar R_F values to N651 and five bands similar to those of N650. Strain N650 fits well in group I (Table 3) with strains of subgroup 14A, N38/14D (*N. opaca*) and Lspi strains N651 and N653. An SPD value of 0.93 between N650 and rhodochrous strain N420 (*N. pellegrino*) suggested a close antigenic relationship. The PAGE patterns of these two strains were similar (Fig. 1, group I) and there were at least 15 corresponding bands (Table 4). Strain N653 (Lspi) shared an SPD value of 0.5 with strain N361 (*N. rubra*) and their PAGE patterns had seven corresponding bands. Strain N651 (Lspi) shared low SPD values with strains N99 (*N. asteroides*) from cluster 1 (-0.88), and N5 (*N. salmonicolor*) (1.5) and N54 (*M. rhodochrous* (-1.0) from subgroup 14C (Goodfellow, 1971). R_F values indicate possible identity of seven bands of strains N651

Fig. 1. PAGE patterns of 17 rhodochrous and allied strains, indicating similarities in band patterns. Identification of the more prominent bands by number is given to the right of each band. Groups I to IV indicate gels with an identical numbering system.

Table 4. Relative mobilities (R_F) of prominent bands in the PAGE patterns of 17 rhodochrous and allied strains

Group I																
Band ...	I	a	b	2	3	4	5	6	c	7	8	9	10	11	d	12
N124/14E (<i>N. opaca</i>)	0.10	—	—	—	0.26	—	0.43	0.45	0.49	—	0.56	—	—	—	0.67	0.69
N324/14A	0.10	—	—	—	0.26	0.29	0.43	0.45	0.49	0.52	0.56	—	0.61	0.64	0.67	0.69
(<i>N. pellegrino</i>)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
N361 (<i>N. rubra</i>)	—	—	—	0.24	0.26	0.29	0.42	0.46	—	0.52	0.56	0.58	—	—	—	0.68
N325/14A	0.10	—	—	0.24	0.26	0.29	0.43	0.46	—	0.52	0.56	0.59	—	0.64	—	0.68
(<i>N. pellegrino</i>)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
N420/14A	0.09	—	—	0.24	0.26	0.29	0.42	0.46	—	0.52	0.56	0.58	0.61	—	—	0.68
(<i>N. pellegrino</i>)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
N650 (Lspi)	0.09	—	—	0.24	0.26	0.29	0.42	0.46	—	0.52	0.56	0.58	0.61	0.63	—	0.68
N653 (Lspi)	0.09	0.10	—	0.24	0.26	—	0.42	0.45	—	—	0.56	0.58	—	0.63	—	0.68
N652 (Lspi)	0.09	0.10	0.13	—	—	—	—	—	—	—	—	0.58	—	0.64	—	—
N651 (Lspi)	—	—	0.13	—	—	—	0.42	0.46	—	—	0.56	—	—	0.63	—	0.69
N99 (<i>N. asteroides</i>)	0.09	0.10	—	—	—	—	0.42	—	—	—	—	0.58	—	0.64	—	—
Group II																
Band ...	I	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
N5/14C (<i>N. salmonicolor</i>)	0.26	0.36	—	—	—	—	—	—	—	—	—	—	—	—	—	—
N54/14C (<i>M. rhodochrous</i>)	0.27	0.36	0.46	0.46	0.46	0.46	0.51	0.51	0.53	0.53	0.64	0.64	0.69	0.69	0.78	0.78
Group III																
Band ...	I	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
N38/14D (<i>N. opaca</i>)	—	—	0.14	0.21	—	—	0.30	0.38	0.45	0.48	0.56	0.59	0.66	0.72	0.77	0.80
N53/14D (<i>J. canicruria</i>)	0.04	0.08	0.14	—	0.23	0.28	0.30	0.38	0.45	0.48	0.56	0.59	0.66	0.72	0.77	0.80
N41 (<i>N. calcarea</i>)	0.05	0.09	0.14	—	0.23	0.28	0.30	0.38	0.45	0.48	0.56	0.59	0.66	0.73	0.77	0.80
Group IV																
Band ...	I	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
N656 (<i>G. terrae</i>)	0.10	0.19	0.24	0.34	0.45	0.51	0.54	0.61	0.67	0.76	0.83	—	—	—	—	—
N655 (<i>G. rubra</i>)	0.10	0.19	0.24	0.34	0.45	0.51	0.54	0.61	0.67	0.76	0.83	—	—	—	—	—

Relative mobility (R_F) is defined as the absolute mobility of the band divided by the mobility of the tracking dye. For the purposes of comparison, the 17 organisms have been separated into four groups, each with a different numbering system for the bands, as shown in Fig. 1. Similar R_F values within each group correspond to the same band number, but do not necessarily correspond to band numbers in any other group. Not all bands for each organism are shown.

and N5 (0.38, 0.42, 0.46, 0.51, 0.63, 0.64 and 0.69), and six bands of strains N651 and N54 (0.11, 0.46, 0.51, 0.56, 0.63 and 0.69). Not all of the R_F values are shown in Table 4.

Strain N99 (*N. asteroides*), except for a small SPD shared with strain N651, was not closely related to any other strain. SPD values ranged from 4.7 to 12.6, with an average of 8.5, which supports the suggestion that the 'rhodochrous' complex is distinct from the *Nocardia* group (Goodfellow *et al.*, 1974). The seeming discrepancy in the low SPD value between two distantly related organisms such as strains N99 and N651 is probably due to dominant skin reactive antigens shared by both species. Each skin reaction is the summation of the interaction between a number of antigens interacting with specifically sensitized lymphocytes. When two organisms share a large number of antigens, the relationship usually results in a low SPD value. Occasionally, as in the present instance, organisms which are apparently distantly related share a low SPD. If such organisms share a few antigens towards which sensitivity may be strong and predominant, this could result in the induction of mutually large skin test reactions and a low SPD. Although only four antigens were matched between strains N99 and N651, these may be predominant shared antigens that account for the skin reactivity. However, other antigens may be involved even though they are not matched by PAGE patterns. Confirmation of this suggestion can only be made by isolating such antigens and by demonstrating their antigenic similarity. The Weil-Felix reaction (Davis *et al.*, 1969) is an example of similar antigens being produced by two distantly related organisms, the *Rickettsia* and *Proteus* species.

Organisms of the genus *Gordona* were originally isolated from sputa of patients with pulmonary disease and from soil. Biochemical, nutritional and morphological tests and the degree of acid fastness provided criteria by which the *Gordona* were placed between the genera *Nocardia* and *Mycobacterium*. *Gordona* species were found to be most closely related to mycobacteria, differing in only one or two characteristics, and Tsukamura (1971) proposed that certain *M. rhodochrous* strains be classified in the genus *Gordona*. Our findings do not support this proposal. The specificity differences with the rest of the complex were high overall (Table 3), with the exception of strain N656 (*Gordona terrae*) and Lspi strain N650, and suggested that the two *Gordona* species tested are distinct and share few antigenic determinants with the rhodochrous organisms. *Gordona* and rhodochrous strains have also been distinguished on the basis of differences in their mycolic acids (Alshamaony, Goodfellow & Minnikin, 1976) and antibiotic sensitivity patterns (Goodfellow & Orchard, 1974). The relatedness of the two *Gordona* species was supported by the PAGE patterns, which indicated at least 11 corresponding bands (Fig. 1, group IV).

The taxonomic structure of the 'rhodochrous' complex is emerging. Relatedness among organisms has largely been determined by morphology, biochemical activity, temperature requirements and cultural appearance. Various immunological techniques have been found to be of taxonomic value at the generic, subgeneric, subspecific and infraspecific levels (Runyon, 1959; Schaefer, 1965; Stanford & Beck, 1968; Magnusson, 1973; Stanford & Grange, 1974). In the present studies, antigenic relatedness was assessed by the ability of extracts to induce skin reactions in sensitized guinea pigs. The results can be used to support some earlier groupings which had been proposed on the basis of biochemical, nutritional and morphological criteria. The PAGE patterns, although also not specific, offered a preliminary glimpse of presumptive antigenic identity. Since every protein has the potential of being an antigen, it is not unreasonable to assume that protein staining bands are antigens, or at least haptens. When two or more isolates shared specificity differences of 1 or less, there was good correlation with the PAGE studies in which similar patterns in appearance and band migration were observed. Studies are currently in progress to define more accurately, and with specific antisera, the degree of relatedness within this complex group.

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