Deoxyribonucleic Acid Reassociation in the Classification of the 'rhodochrous' Complex and Allied Taxa

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SUMMARY

The degree of binding was determined between DNA preparations from gordonae and rhodochrous strains and uracil-labelled DNA from five reference strains, *Nocardia asteroides* NK20, *N. pellegrino* P11, *Gordona bronchialis* T1, *G. terrae* T5 and rhodochrous strain R90. Most of the rhodochrous and pellegrino strains fell into one of two genetically homogeneous taxa. The nucleotide sequence homology data also suggested that *G. bronchialis*, *G. rubra*, and more equivocally *G. terrae*, formed distinct species. However, the values for DNA relatedness between these species, and between them and the rhodochrous homology groups, were comparatively low. Only a small degree of nucleotide sequence homology was found between the *N. asteroides* reference system and the rest of the taxa studied. The nucleotide composition of the DNA preparations from 29 of the 30 test strains fell between 63 and 69 mol % guanine plus cytosine.

INTRODUCTION

An increasing volume of taxonomic data supports the view that the 'rhodochrous' complex should be considered as a taxon equivalent in rank to the genera *Nocardia* and *Mycobacterium* (Cross & Goodfellow, 1973). Much of the data comes from numerical phenetic studies (Bradley, 1971; Goodfellow, 1971; Tacquet *et al.*, 1971; Goodfellow, Fleming & Sackin, 1972; Jones, 1975) although additional evidence is provided by chemical and serological analyses (Ridell & Norlin, 1973; Alshamaony, Goodfellow & Minnikin, 1976a). Recently, in an extensive investigation of rhodochrous and related strains, carried out under the auspices of the International Working Group on Mycobacterial Taxonomy, further concordance was found between phenetic, serological and chemical data (Goodfellow *et al.*, 1974). Representative strains of the proposed genus *Gordona* (Tsukamura, 1971) were not included in any of these studies and the relationship between this taxon and the 'rhodochrous' complex is not settled. However, on the basis of numerical phenetic data, Tsukamura (1973, 1974) classified a number of rhodochrous strains in the genus *Gordona*.

In all the numerical studies, the rhodochrous strains formed a distinct but heterogeneous

taxon. In some instances the strains fell into two clusters (Bradley, 1971; Tacquet *et al.*, 1971; Jones, 1975) but in others three or more homogeneous phena were recovered (Goodfellow, 1971; Goodfellow *et al.*, 1974). Since few of the cultures examined were common to all of the investigations it is difficult to determine whether or not, and to what extent, phena overlap. It is evident, however, that *Nocardia rubra* (Bradley, 1971) corresponds at least partially with phenon 14C (Goodfellow, 1971), *Gordona rhodochroa* (Tsukamura, 1973) with phenon 1a (Goodfellow *et al.*, 1974), and *Nocardia erythropolis* (Bradley, 1971) can be equated with phenon 14D (Goodfellow, 1971) and phenon F3 (Jones, 1975). Further evidence of the heterogeneity of the 'rhodochrous' complex can be drawn from genetic recombination (Adams, Adams & Brownell, 1970), DNA:DNA pairing (Bradley & Huitron, 1973; Bradley, Brownell & Clark, 1973), serological (Ridell, 1974) and chemical data (Goodfellow *et al.*, 1974; Alshamaony *et al.*, 1976b).

In view of the uncertain taxonomy of the 'rhodochrous' complex and related taxa, we have undertaken DNA reassociation assays on such nocardioform bacteria. This preliminary report describes the determination of the degree of binding between DNA preparations from rhodochrous and *Gordona* strains and uracil-labelled DNA from five reference strains.

METHODS

Test strains and growth conditions. Details of the strains and their sources are given in Table 1. All cultures were maintained routinely on yeast extract agar at room temperature.

For DNA extraction, organisms were grown in submerged culture in modified Sauton's medium (Mordarska, Mordarski & Goodfellow, 1972) or in peptone yeast extract broth (Bradley, 1973) for 30 to 40 h at 37 °C. Cultures were checked for purity; and then the organisms were harvested by centrifuging, washed in saline ethylenediaminetetraacetate (0.15 M-NaCl, 0.1 M-EDTA, pH 8.0) and stored at -20 °C.

Extraction of DNA. Harvested organisms (200 mg wet wt) were suspended in 1 ml of buffer A [0.015 M-Tris-HCl (pH 8.0), 0.45 M-sucrose and 0.008 M-EDTA] containing 1 mg lysozyme (Sigma or Serva)/ml and incubated for 18 h at 37 °C. The organisms were then separated by centrifuging, and resuspended in 0.5 ml of buffer B [0.1 M-Tris-HCl (pH 9.0), 0.01 M-NaCl and 1 mM-sodium citrate]. Strains not lysed by lysozyme were treated for 90 s in a Brown disintegrator; if the lysate was considered to be dense, a little more buffer B was added.

After lysis, sodium dodecyl sulphate (SDS, 2%, w/v) was added and the suspension heated for 30 min at 60 °C, rapidly cooled and an equal volume of freshly-distilled phenol saturated with Tris-SDS buffer (0.01 M-Tris, 1%, w/v, SDS and 0.1 M-NaCl, pH 9.0) added (Saito & Miura, 1963). The resulting mixture was shaken in a cooled gyratory incubator for 20 min and the emulsion formed was separated in a cooled centrifuge (10 min, 10000 g). The aqueous phase was removed and treated twice again with an equal volume of phenol saturated with the Tris-SDS buffer. Nucleic acids were precipitated from the aqueous fraction by the addition of 2 vols of cooled ethanol (-10 °C). The precipitate was spooled on a glass rod, dissolved in 0.1 × SSC (SSC: 0.15 M-NaCl, 0.015 M-trisodium citrate), and the salt concentration increased to 1 × SSC by the addition of 10 × SSC. This solution was incubated at 37 °C for 30 min with 50 µg pancreatic ribonuclease (Sigma)/ml and pre-incubated at 100 °C for 10 min, to destroy DNAase.

After digestion, an equal volume of phenol saturated with water was added and the mixture shaken for 10 min in a refrigerator. The resulting emulsion was centrifuged and the aqueous phase separated. Phenol extraction was repeated several times, usually three, until

Table 1. Designation, source and DNA base composition of test strains

Strain	GC (mol %)	Source	Strain	GC (mol %)	Source
<i>Gordona bronchialis</i> T1	65	M. Tsukamura, Chuba Chest Hospital, Obu, Aichi-ken 474, Japan, 3410. Type strain. Sputum. ATCC25592. NCTC10667	<i>Nocardia pellegrino</i> P1	68	Institut für experimentelle Biologie und Medizin, Borstel, BDR, sn5101
<i>G. bronchialis</i> T2	63	M. Tsukamura. Sputum	<i>N. pellegrino</i> P2	68	sn5102
<i>Gordona rubra</i> T3	67	M. Tsukamura, 3605. Soil. ATCC25593. NCTC10668	<i>N. pellegrino</i> P3	68	sn5103
<i>G. rubra</i> T4	67	M. Tsukamura. Soil. ATCC60004	<i>N. pellegrino</i> P4	66	sn5104
<i>Gordona terrae</i> T5	64	M. Tsukamura, 3612. Soil. ATCC25594. NCTC10669	<i>N. pellegrino</i> P5	68	sn5105
<i>G. terrae</i> T6	69	M. Tsukamura. Soil.	<i>N. pellegrino</i> P6	77	sn5106
<i>Nocardia asteroides</i> NK20	68	ATCC3308	<i>N. pellegrino</i> P7	68	sn5107
			<i>N. pellegrino</i> P8	69	sn5108
			<i>N. pellegrino</i> P9	68	sn5110
			<i>N. pellegrino</i> P10	65	sn5111
			<i>N. pellegrino</i> P11	68	sn5112
			'rhodochrous' R3	66	ATCC999
			'rhodochrous' R5	69	ATCC4004
			'rhodochrous' R10	65	ATCC13808
			'rhodochrous' R14	67	ATCC14348
			'rhodochrous' R35	64	ATCC25671
			'rhodochrous' R61	66	ATCC25700
			'rhodochrous' R70	64	ATCC25709
			'rhodochrous' R73	65	ATCC25712
			'rhodochrous' R80	63	ATCC25722
			'rhodochrous' R83	67	ATCC25725
			'rhodochrous' R84	66	ATCC25726
			'rhodochrous' R90	66	ATCC25732

ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A. NCTC, National Collection of Type Cultures, London.

the interphase layer disappeared. The purified DNA was precipitated with cooled ethanol, redissolved in $0.1 \times \text{SSC}$, and 0.1 vol. of 3.0 M -sodium acetate- 0.001 M -EDTA solution, pH 7.0, was added. The DNA was then precipitated by slowly adding 0.54 vol. of isopropanol, wound on a glass rod, and washed in ethanol solutions of increasing concentration (70, 80 and 90%). After drying, the DNA was redissolved in $0.1 \times \text{SSC}$ and the treatment with isopropanol repeated until a preparation having E_{260}/E_{280} of 1.9 to 2.0 was obtained, and E_{230}/E_{255} was greater than 2. The purified DNA was stored at 5°C in $0.1 \times \text{SSC}$ containing a few drops of chloroform.

Preparation of radioactive DNA for homology studies. [$2\text{-}^{14}\text{C}$]Uracil (Nuclear Research Institute, Swierk, Poland), specific activity 9.6 to 12.3 mCi/mmol , was added to inoculated media to give a final concentration of $1 \mu\text{Ci/ml}$. Labelled organisms were centrifuged and the DNA extracted and purified as described. Sheared labelled DNA was prepared by sonication in an ice bath at a frequency of 24 kHz (six periods of 15 s with 30 s intervals).

Labelled reference DNA was prepared from five strains: *Gordona bronchialis* T1 (ATCC 25592), *G. terrae* T5 (ATCC25594), *Nocardia asteroides* NK20 (ATCC3308), *N. pellegrino* P11 and the rhodochrous strain R90 (ATCC25732). The specific activities of the reference DNA preparations are shown in Table 2.

Analysis of base compositions. The mol % guanine plus cytosine was determined by the method of Huang & Rosenberg (1966).

Fixation of denatured, high molecular weight DNA on membrane filters. The relationships

between the test strains were established by determining the extent to which single-stranded DNA, immobilized on nitrocellulose membrane filters, bound labelled reference DNA which was in solution. The membrane filter technique of Legault-Demare *et al.* (1967), as modified by De Ley & Tijtgaat (1970), was used. The ratio of labelled to unlabelled DNA was 1:15.

High molecular weight DNA in $0.1 \times \text{SSC}$ ($150 \mu\text{g/ml}$) was denatured by boiling for 10 min at 100°C , cooled rapidly in an ice bath, and the salt concentration adjusted to $6 \times \text{SSC}$. Immobilized DNA (150 mg) was prepared by allowing the heat denatured DNA solution to filter by gravity through nitrocellulose filter discs (Sartorius 25 mm SM1140 , pore size $0.45 \mu\text{m}$). The filters were then washed with 50 ml of $6 \times \text{SSC}$, dried at room temperature for 24 h, and placed in a vacuum oven at 80°C for 2 h.

DNA:DNA pairing. Filters containing the denatured, unlabelled test DNA were placed in small bottles containing $10 \mu\text{g}$ sheared, denatured labelled reference DNA in 2 ml of $2 \times \text{SSC}$ in 30% (v/v) dimethylsulphoxide (DMSO). After incubation for 18 h at 65°C the filters were removed and washed with 50 ml of $2 \times \text{SSC}$ -DMSO, then with 50 ml of $2 \times \text{SSC}$. The dried filters were examined for bound radioactivity in a liquid scintillation counter (Nuclear, Chicago) using a scintillation fluid consisting of 1 l toluene, 8 g 2,5-diphenyl-oxazole (PPO) and 200 mg 2,2'-*p*-phenylen-bis-(4-methyl-5-phenyloxazol) [POPOP].

Thermal stability of DNA:DNA duplexes. The thermal stability of duplexes formed between filter-bound DNA and the various reference DNA preparations was determined from the $T_{m(e)}$ values. The midpoint of the thermal elution ($T_{m(e)}$) is the temperature at which half of the reassociated reference DNA becomes dissociated and eluted from the test DNA bound to the filter. $\Delta T_{m(e)}$ was found by subtracting $T_{m(e)}$ of hybrids of the heterologous system from $T_{m(e)}$ of homologous hybrids (Okaniski & Gregory, 1970).

After pairing, the filters were dried and then eluted with $0.1 \times \text{SSC}$ solution at temperature increments of 5°C over the range 70 to 100°C . The radioactive samples were assayed in the scintillation fluid of Bray (1960).

RESULTS

DNA base compositions

The nucleotide composition of the DNA preparations from 29 of the 30 test strains fell within the range 63 to 69 mol % guanine (G) plus cytosine (C). *Nocardia pellegrino* P6, showed a high GC content of 77%.

DNA:DNA pairing

The DNA preparations from 9 of the 10 strains received as *N. pellegrino* bound significant amounts of reference DNA from *N. pellegrino* P11 but showed little affinity with that from *N. asteroides* NK20, *G. bronchialis* T1 and *G. terrae* T5 (Table 2). The remaining strain, *N. pellegrino* P6 shared little homology with the *N. pellegrino* reference strain but bound 80% of the reference DNA from the rhodochrous strain R90. A much smaller degree of nucleotide sequence homology (15 to 30%) was found between the other *N. pellegrino* strains and the rhodochrous reference strain (Table 2). A typical elution curve of a DNA duplex formed between reference *N. pellegrino* DNA and DNA of a *N. pellegrino* strain is shown in Fig. 1.

The DNA preparations of 11 of the 12 rhodochrous strains bound 60 to 100% of the reference DNA from the rhodochrous strain R90, but with few exceptions showed a low degree of homology with the other reference systems (Table 3). The remaining rhodochrous culture, R35, showed little nucleotide homology with any of the reference systems. However, very high nucleotide sequence homologies were found between the reference systems and

Table 2. Reassociation of DNA samples from *Nocardia pellegrino* strains with the DNA from reference strains *N. pellegrino* P11, *N. asteroides* NK20, *Gordona bronchialis* T1, *G. terrae* T5 and 'rhodochrous' R90

The extent of binding between DNA from test strains and labelled DNA from reference strains is expressed as a percentage relative to the extent of binding between labelled and unlabelled DNA from the reference strain itself. $\Delta T_{m(e)}$, a measure of the thermal stability of the DNA : DNA duplexes, was obtained by subtracting $T_{m(e)}$, the midpoint of thermal elution, of the molecular hybrids of the heterologous system from the $T_{m(e)}$ of the homologous hybrids. For details of measurement, see Methods.

DNA source	Labelled DNA source								
	<i>N. pellegrino</i> P11		<i>N. asteroides</i> NK20		<i>G. bronchialis</i> T1	<i>G. terrae</i> T5		'rhodochrous' R90	
	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)
<i>N. pellegrino</i> P11	100	0	13	—	16±4	12±2	3	29±0	3
<i>N. pellegrino</i> P1	102±4	0	16	0	16±2	11±1	—	27±2	10
<i>N. pellegrino</i> P2	82±2	9	12	0	11±1	11±2	—	20±1	17
<i>N. pellegrino</i> P3	101±6	15	15	0	15±2	12±0	7	26±1	—
<i>N. pellegrino</i> P4	104±0	14	15	9	14±1	17±3	—	30±1	0
<i>N. pellegrino</i> P5	63±1	7	10	0	13±1	10±1	14	22±1	16
<i>N. pellegrino</i> P6	12±1	3	15	0	16±2	14±2	1	81±10	10
<i>N. pellegrino</i> P7	89±2	12	15	—	14±1	15±3	10	27±2	—
<i>N. pellegrino</i> P8	96±3	10	11	—	21±2	10±0	—	22±1	—
<i>N. pellegrino</i> P9	82±0	—	10	—	9±0	7±1	9	20±0	—
<i>N. pellegrino</i> P10	104±4	10	17	—	17±1	15±2	—	26±1	—
<i>N. asteroides</i> NK20	14	—	100	—	11±0	12±1	—	13	—
<i>G. bronchialis</i> T1	15±1	2	11	0	100	15±3	—	15±1	—
<i>G. terrae</i> T5	15±2	12	13	4	21±4	100	—	28±2	—
'rhodochrous' R90	31±3	14	9	0	13	11±1	—	100	—

Specific activities of the DNA samples from reference strains were (c.p.m./μg): *N. pellegrino* P11, 3445; *N. asteroides* NK20, 6890; *G. bronchialis* T1, 5578; *G. terrae* T5, 3114; 'rhodochrous' R90, 2764.

Table 3. Reassociation of DNA samples from rhodochrous strains with DNA from the reference strains 'rhodochrous' R90, *Nocardia asteroides* NK20, *N. pellegrino* P11, *Gordona bronchialis* T1 and *G. terrae* T5

DNA source	Labelled DNA source								
	'rhodochrous' R90		<i>N. asteroides</i> NK20		<i>N. pellegrino</i> P11		<i>G. bronchialis</i> T1	<i>G. terrae</i> T5	
	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)
'rhodochrous' R3	84±0	—	11	0	22	7	15±1	15±3	0
'rhodochrous' R5	> 120	17	53	—	> 120	—	> 120	> 120	—
'rhodochrous' R10	90±0	11	8	0	14±5	5	10±0	12±1	8
'rhodochrous' R14	112±2	—	9	4	14±5	8	10±1	15±3	0
'rhodochrous' R35	8±3	—	6	0	16	—	14±1	18±5	0
'rhodochrous' R61	95±5	2	12	0	15±3	0	7±1	15±0	—
'rhodochrous' R70	82±2	—	12	—	11±3	—	11±0	19±5	0
'rhodochrous' R73	102±6	—	12	—	11±2	8	15±0	13±1	12
'rhodochrous' R80	60	3	15	4	25	—	11	15	7
'rhodochrous' R83	73±1	—	11	—	22±8	14	22±1	28±1	—
'rhodochrous' R84	85±3	—	10	3	27±2	—	12±2	22±0	—
'rhodochrous' R90	100	—	9	0	31±3	14	11±1	13±1	0
<i>N. asteroides</i> NK20	13	—	100	0	14	—	11±1	12±1	—
<i>N. pellegrino</i> P11	29±0	—	13	—	100	—	12±2	16±4	—
<i>G. terrae</i> T5	28±2	16	13	0	15±2	—	100	21±4	0
<i>G. terrae</i> T1	15±1	—	11	0	15±1	—	15±3	100	—

For definition of $\Delta T_{m(e)}$, and specific activities of DNA from the reference strains, see Table 2.

Table 4. Reassociation of DNA samples from *Gordona* strains with DNA from the reference strains *G. bronchialis* T1, *G. terrae* T5, *Nocardia asteroides* NK20, *N. pellegrino* P11 and 'rhodochrous' R90

DNA source	Labelled DNA source								
	<i>G. bronchialis</i> T1	<i>G. terrae</i> T5		<i>N. asteroides</i> NK20		<i>N. pellegrino</i> P11		'rhodochrous' R90	
	Rel. DNA bound (%)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)	Rel. DNA bound (%)	$\Delta T_{m(e)}$ (°C)
<i>G. bronchialis</i> T1	100	15±3	0	11	0	15±1	2	15±1	—
<i>G. bronchialis</i> T2	100	22±0	0	11	—	12±3	—	11±0	—
<i>G. rubra</i> T3	25	18±5	0	13	—	9±1	—	9±0	—
<i>G. rubra</i> T4	22±1	18±1	0	13	0	15±2	2	14±1	12
<i>G. terrae</i> T5	21±4	100	0	13	4	15±2	12	28±2	16
<i>G. terrae</i> T6	16±3	44±3	0	4	0	9±0	10	8±0	22
<i>N. asteroides</i> NK20	11±1	12±1	—	100	0	14	—	13	—
<i>N. pellegrino</i> P11	12±2	16±4	3	13	—	100	0	29±0	—
'rhodochrous' R90	11±1	13	—	9	0	31±3	14	100	—

For definition of $\Delta T_{m(e)}$, and specific activities of DNA from the reference strains, see Table 2.

Fig. 1

Fig. 2

Fig. 1. Thermal elution profile of DNA:DNA duplexes of *N. pellegrino* P11 and *N. pellegrino* P10. See Methods for details.

Fig. 2. Thermal elution profile of homologous DNA:DNA duplexes of *Gordona terrae* T5. See Methods for details.

rhodochrous strain R5. Five of the rhodochrous strains bound between 22 and 31% of reference *N. pellegrino* DNA and an equivalent affinity was found between some of these strains and the *G. bronchialis* and *G. terrae* reference systems (Table 3).

A low degree of homology was exhibited between DNA preparations from the *Gordona* strains and DNA from *N. asteroides* NK20, *N. pellegrino* P11 and the rhodochrous strain R90 (Table 4). The highest affinity was the 28% nucleotide sequence homology found between *G. terrae* T5 and the rhodochrous reference system. Similarly comparatively little affinity was exhibited between the DNA from *G. rubra* and *G. terrae* and that from the *G. bronchialis* reference system. The two *G. bronchialis* strains, however, shared a similarity of 100%. In contrast, *G. terrae* T6 only bound 44% of the DNA from the *G. terrae* reference system. The thermal elution profile of the homologous DNA:DNA duplex of *G. terrae* T5 is shown in Fig. 2.

In most of the reassociation experiments the thermal stability of the molecular hybrids was similar to those formed in the homologous systems, although lower thermal stabilities of heterologous DNA duplexes were often noted.

DISCUSSION

Good congruence has been observed between nucleotide sequence homology and numerical phenetic data in a number of genera (Sneath, 1972; Kersters *et al.*, 1973; Colwell *et al.*, 1974; Staley & Colwell, 1973) and in some actinomycete taxa (Gross & Wayne, 1970; Farina & Bradley, 1970; Okanishi & Gregory, 1970; Bradley, 1972). In most cases, strains classified in a homogeneous species share at least 70% DNA, and lower binding values are considered to reflect significant genetic divergence (Brenner, 1973). However, it is still difficult to equate homology and similarity values, or to formally relate either of them to levels in the taxonomic hierarchy. Similarly coefficients can be severely distorted by factors

such as sampling error, poor test reproducibility and failure to account for differences in growth rate and metabolic activity. On the other hand, the techniques used to determine DNA homology are complex and may not be free from significant experimental errors. Our estimates of genetic resemblance should not, therefore, be regarded as absolute values.

In some of the experiments described, large amounts of labelled DNA were needed, for instance, with the rhodochrous strain R90 the specific activity of the reference DNA was 2764 c.p.m./ μ g. However, using 25 mm discs, sufficient labelled DNA was bound for reproducibility despite the low specific activity. In the homologous systems 20 to 30% pairing (3500 to 18000 c.p.m./sample) was found and taken as 100%.

Only a small degree of genetic relatedness existed between the *Nocardia asteroides* reference system and the rest of the taxa studied. Although only one *Nocardia* strain was examined the results support numerical, chemical and serological studies (Ridell, 1974; Goodfellow *et al.*, 1974; Alshamaony *et al.*, 1976*a*) which suggest that the 'rhodochrous' complex should not be classified in the genus *Nocardia sensu stricto* (Cross & Goodfellow, 1973). They also support the view (Tsukamura, 1971) that the genera *Gordona* and *Nocardia* are related but distinct.

The internal structure of the 'rhodochrous' complex is gradually being revealed but it is still difficult to draw boundaries between the various clusters recovered (Goodfellow, 1971; Goodfellow *et al.*, 1974). It was, therefore, encouraging that nearly all of the representatives of phenon 1*a* (Goodfellow *et al.*, 1974), the *N. rubra* taxon (Bradley, 1971), formed a tightly knit group in which the strains shared 80% or more DNA with the rhodochrous reference system. However, the DNA base composition range of the constituent strains, 63 to 69% GC, was wider than that reported for *N. rubra* (Bradley, 1971). Nevertheless, 1*a* forms a good cluster on the basis of numerical phenetic, chemical, serological (Goodfellow *et al.*, 1974; Alshamaony *et al.*, 1976*a*) and genetic data. The only representative of the rhodochrous phenon 1*b* (Goodfellow *et al.*, 1974), rhodochrous strain R35, shared little genetic affinity with any of the reference strains studied. Additional representatives of phenon 1*b* need to be examined to see whether they share a high DNA homology with reference DNA from strain R35.

The strains labelled *N. pellegrino* form a homogeneous taxon on the basis of chemical and fermentation studies (Mordarska, Mordarski & Pietkiewicz, 1973), and there is strong evidence that they also comprise a good cluster in the 'rhodochrous' complex (M. Goodfellow, unpublished data). This *N. pellegrino* taxon forms a genetically homogeneous group with all but one of the strains showing 82% or more binding with DNA from the reference pellegrino strains. Values of DNA relatedness ranging from 20 to 30% were obtained between the rhodochrous reference strains and the pellegrino strains. The pellegrino strains exhibited a DNA base composition range of 66 to 69% GC so they cannot be differentiated from the other rhodochrous strains on this basis.

The nucleotide sequence homology data suggested that *G. bronchialis*, *G. rubra*, and more equivocally *G. terrae*, formed good species. However, the values for DNA relatedness between these three taxa, and between them and the rhodochrous homology groups, were comparatively low. *Gordonae* have also been distinguished from rhodochrous strains in antibiotic sensitivity studies (Goodfellow & Orchard, 1974) and in lipid analyses (Alshamaony *et al.*, 1976*b*). On the basis of numerical phenetic data, Tsukamura (1973, 1974) has classified a number of rhodochrous strains in new species within the genus *Gordona*. The DNA base composition range of the *gordonae* is similar to that shown by *nocardiae*, *mycobacteria* and rhodochrous strains.

Clearly more extensive numerical and nucleotide sequence homology analyses are required to unravel the complicated taxonomic relationships between *Gordona* and rhodochrous clusters, and our studies are being continued.

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