Ribosomal Ribonucleic Acid Similarities in the Classification of *Rhodococcus* and Related Taxa

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(Received 3 December 1979)

Duplexes were prepared between ¹⁴C-labelled rRNA from both *Rhodococcus equi* C7 and *Rhodococcus rhodochrous* N54 and DNA from 16 actinomycetes representing the genera *Rhodococcus*, *Mycobacterium*, *Nocardia*, *Saccharopolyspora* and *Streptomyces*. The relationships between the organisms were determined by plotting the temperature at which 50 % of the duplex was denatured ($T_{m(50)}$) against the percentage of rRNA binding (μg ¹⁴C-labelled rRNA duplexed per 100 μg filter-bound DNA). All of the strains formed stable duplexes but each organism occupied a definite area on the rRNA similarity map. All of the organisms share a close phylogenetic relationship but representatives of the genera *Rhodococcus*, *Mycobacterium*, *Nocardia* and *Streptomyces* fell into four recognizable clusters on the similarity map. These data support and extend current trends in the classification of *Rhodococcus* and allied taxa. The guanine plus cytosine content of the DNA from the test strains was within the range 69.3 to 76.9 mol %.

INTRODUCTION

The genus *Rhodococcus* (Zopf, 1891) was reintroduced by Goodfellow & Alderson (1977) to accommodate certain actinomycetes previously classified in genera such as *Corynebacterium*, *Gordona*, *Jensenia*, *Mycobacterium* and *Nocardia*. Numerical phenetic surveys and chemotaxonomic studies have led to significant improvements in the classification of *Rhodococcus*, *Corynebacterium*, *Mycobacterium* and *Nocardia* (Goodfellow & Minnikin, 1977, 1978; Jones, 1975, 1978; Keddle & Cure, 1978; Minnikin & Goodfellow, 1976, 1980; Minnikin *et al.*, 1978) and to the recognition of the '*aurantiaca*' taxon (Goodfellow *et al.*, 1978) but have shed little light on the evolutionary relationships of these taxa. The chemical evidence indicates that these taxa are closely related, as representative strains contain mycolic acids, have similar fatty acid and polar lipid compositions (Minnikin & Goodfellow, 1980; Minnikin *et al.*, 1978) and possess a *meso*-diaminopimelic acid-containing peptidoglycan associated with an arabinogalactan (wall chemotype IV; Lechevalier & Lechevalier, 1970). Also, the guanine plus cytosine content of the DNA from rhodococci, mycobacteria and nocardiae is relatively high (> 59 mol % GC) and can be distinguished from that of true corynebacteria (48 to 59 mol % GC; see, for example, Crombach, 1978; Minnikin *et al.*, 1978; Mordarski *et al.*, 1978).

The 10 species in the genus *Rhodococcus* were recognized primarily on the results of numerical phenetic and chemical studies (Goodfellow & Alderson, 1977; Rowbotham &

Cross, 1977). DNA:DNA pairing data support the numerical classification but are of little value in establishing relationships between *Rhodococcus* species, or between these species and those of related taxa, as strains in the various homology groups have little DNA in common (Bradley & Mordarski, 1976; Mordarski *et al.*, 1976, 1977, 1978, 1980). Clearly, additional taxonomic methods are required to determine the phylogenetic relationships between such genetically diverse taxa.

It is now well established that the base sequences of rRNA cistrons are more highly conserved than most of the genes forming the bacterial genome (Doi & Igarashi, 1965; Dubnau *et al.*, 1965; Moore & McCarthy, 1967; Takahashi *et al.*, 1967) and that similarities in nucleotide sequences of rRNA preparations provide a means of establishing relationships among representatives of diverse bacterial taxa (Moore & McCarthy, 1967, 1969; Johnson *et al.*, 1970; Pace & Campbell, 1971*a, b*; De Smedt & De Ley, 1977). Such studies have also been invaluable in clarifying the structure of large and heterogeneous taxa such as *Clostridium* (Johnson & Francis, 1975) and *Pseudomonas* (Palleroni *et al.*, 1973).

The present experiments on rRNA:DNA pairing were designed to examine the homogeneity of the genus *Rhodococcus* and its evolutionary relationships with the genera *Mycobacterium*, *Nocardia*, *Saccharopolyspora* and *Streptomyces*.

METHODS

Test strains and growth conditions. The strains and their sources are listed in Table 1. More detailed histories of strains can be found elsewhere (Goodfellow, 1971; Goodfellow & Alderson, 1977; Shirling & Gottlieb, 1968, 1972). The cultivation conditions, harvesting procedure and methods of isolating purified DNA have been described previously (Mordarski *et al.*, 1977).

Preparation of ¹⁴C-labelled rRNA. [2-¹⁴C]Uracil (Nuclear Research Institute, Swierk, Poland; specific activity 310 to 470 GBq mol⁻¹) was added to inoculated modified Sauton's medium to give 74 kBq ml⁻¹. After growth, harvested organisms (20 g wet wt) were washed three times in 60 ml buffer (0.01 M-Tris/HCl, 0.01 M-MgCl₂, 0.01 M-KCl, pH 7.2) and RNA was isolated after Moore & McCarthy (1967). Thus, organisms were suspended in 20 ml buffer (0.01 M-Tris/HCl, 0.01 M-MgCl₂, 0.01 M-KCl, 10 µg DNAase ml⁻¹, pH 7.2) and disrupted by passage through a French pressure cell (140 MPa), or in a Brown disintegrator. In the latter case, the biomass was treated with an RNAase inhibitor, either Bentonite (1 mg ml⁻¹) or polyvinylsulphate (10 µg ml⁻¹), together with DNAase at pH 7.5 before disruption; in the former, the inhibitor was added after disruption. In each case, the resultant mixture was treated with sodium dodecyl sulphate (final concentration 1 %, w/v) and shaken twice at room temperature for 5 min with 1 vol. phenol saturated with water; the RNA was precipitated from the aqueous phase with 2 vol. cold 95 % (v/v) ethanol and collected by centrifugation. The crude RNA preparation was treated with DNAase (10 µg ml⁻¹), incubated at 37 °C for 15 min, treated with Pronase (50 µg ml⁻¹) and incubated for a further 15 min. The mixture was then treated once again with aqueous phenol, centrifuged and the RNA was precipitated with 95 % ethanol. Deproteinization with phenol was repeated three times and the purified RNA was resuspended in SSC buffer (0.15 M-NaCl, 0.015 M-trisodium citrate, pH 7.0).

The purified RNA was fractionated by sucrose gradient centrifugation after McConkey (1967). RNA (about 1 mg) was placed on the surface of a 21 ml sucrose linear gradient [15 to 30 % (w/v) sucrose in 0.1 M-sodium acetate buffer with 0.05 M-NaCl, pH 5 to 5.1] and centrifuged in a Janetzki VAC 60 ultracentrifuge at 108 000 g for 20 h at 4 °C. After centrifugation, samples from each of the collected fractions were assayed for radioactivity to locate the RNA. The 16S and 23S fractions were collected separately, dialysed three times against 4 l of 2 × SSC buffer at 4 °C and stored at -12 °C.

The rRNA:DNA pairing experiments were carried out using the 23S rRNA fractions. Labelled reference rRNA was prepared from *Rhodococcus equi* C7 and *Rhodococcus rhodochrous* N54; the specific activities of the samples were 4161 and 2033 c.p.m. µg⁻¹, respectively.

Preparation of DNA. High molecular weight DNA was prepared using the modification of the method of Saito & Miura (1963) described by Mordarski *et al.* (1976). Single-stranded DNA (30 µg cm⁻²) was immobilized on nitrocellulose membrane filters (Schleicher & Schüll; 50 mm diam.; pore size 0.45 µm).

Analysis of DNA base composition. The mol % GC of DNA preparations was determined by the thermal denaturation method (Marmur & Doty, 1962).

rRNA:DNA pairing and thermal stability of duplexes. The extent of nucleotide pairing and duplex stability were determined after De Ley & De Smedt (1975). ¹⁴C-labelled rRNA (6 µg) was incubated in 1 ml of 2 × SSC in 20 % (v/v) formamide with immobilized DNA (30 µg cm⁻²) for 18 h at 50 °C. After washing, the

Table 1. Test strains used, their source, DNA base composition, and properties of the DNA:rRNA duplexes with two *Rhodococcus* ¹⁴C-labelled rRNA reference systems

Lab. no.	Test organism used for DNA preparation	Source†	Mol % GC	¹⁴ C-labelled rRNA from:			
				<i>R. equi</i> C7		<i>R. rhodochrous</i> N54	
				% binding	<i>T</i> _{m(e)} (°C)	% binding	<i>T</i> _{m(e)} (°C)
*C600	<i>Escherichia coli</i>	NCTC 9001	51.5	0.011	69.5	0.07	74.0
*M377	<i>Mycobacterium chelonae</i>	NCTC 946	72.5	0.06	72.5	0.04	77.0
*M93	<i>M. fortuitum</i>	NCTC 10394	69.5	0.10	75.5	0.08	76.0
*M101	<i>M. smegmatis</i>	NCTC 10265	73.4	0.10	73.5	0.10	76.0
*N667	<i>Nocardia amarae</i>	M.P. Lechevalier, IMRU W3960	71.0	0.22	73.0	0.22	73.0
*N317	<i>N. asteroides</i>	ATCC 19247	74.6	0.24	73.5	0.23	74.5
*N36	<i>N. otitidis-caviarum</i>	NCTC 1934	71.7	0.28	73.5	0.20	76.0
*N744	<i>Rhodococcus coprophilus</i>	NCIB 11211	69.3	0.19	74.0	0.25	76.5
*C7	<i>R. equi</i>	NCTC 1621	70.4	0.23	74.5	ND	ND
N829	<i>R. equi</i> group	R. E. Gordon, 588	70.9	0.23	74.0	0.24	76.5
*N11	<i>R. erythropolis</i>	NCIB 9158	70.6	0.25	74.0	0.20	78.0
*N54	<i>R. rhodochrous</i>	NCIB 11147	69.5	0.22	74.5	0.23	78.5
*N361	<i>R. ruber</i>	M. Tsukamura, M-1	72.3	0.25	77.0	0.27	77.5
*N745	<i>Saccharopolyspora hirsuta</i>	NCIB 11079	76.9	0.21	72.0	0.17	74.5
*N884	<i>Streptomyces griseus</i>	NCTC 3236	74.4	0.14	72.5	0.12	77.8
*N915	<i>S. indigocolor</i>	ISP 5432	74.7	0.18	73.0	0.19	76.5
*N916	<i>S. toyocaensis</i>	ISP 5030	71.0	0.25	72.0	0.20	76.5

ND, Not determined.

* Type strain.

† ATCC, American Type Culture Collection, Rockville, Md, U.S.A.; ISP, International *Streptomyces* Project (see Shirling & Gottlieb, 1968, 1972); NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCTC, National Collection of Type Cultures, London; R. E. Gordon and M. P. Lechevalier, Rutgers University, New Brunswick, N.J., U.S.A.; M. Tsukamura, The National Chubu Hospital, Obu, Aichi, Japan.

membrane filters were treated with RNAase (250 µg) in 2 ml of 2 × SSC buffer at 37 °C for 1 h. The thermal stability of duplexes formed between filter-bound DNA and reference rRNA preparations was determined from the *T*_{m(e)} values, i.e. the temperature at which half of the paired rRNA becomes dissociated and eluted from test DNA bound to the filter. Thus, the thermal stability of duplexes was determined by eluting dried filters with 1.5 × SSC in 20 % (v/v) formamide in 5 °C steps from 60 to 90 °C. The amount of material released at each step was counted in a liquid scintillation counter (Mark 1, Nuclear Chicago) for 50 min. The total amount of bound ¹⁴C-labelled rRNA, expressed as µg rRNA per 100 µg DNA retained on filters, was called the 'percentage of rRNA binding'. The DNA remaining on membranes was released using the method of Meys & Schilperoort (1971) and the amount was determined chemically after Burton (1956).

RESULTS

DNA base compositions. The test strains fell within the range 69.3 to 76.9 mol % GC (Table 1).

rRNA:DNA pairing and thermal stability of duplexes. The ¹⁴C-labelled rRNAs from the reference strains *R. equi* C7 and *R. rhodochrous* N54 were clearly separated on sucrose gradients (Fig. 1). 23S rRNA similarity was expressed both as *T*_{m(e)} and as percentage of rRNA binding (Table 1), as the latter depends not only on the actual rRNA homology but also on the size of the genome, its state of replication and the number of rRNA cistrons in the genome (De Smedt & De Ley, 1977). A few examples of melting point determinations are shown in Fig. 2. The correlation between the two sets of data was revealed by

Fig. 1

Fig. 2

Fig. 1. ^{14}C -labelled rRNA distributions in a linear 15 to 30 % sucrose gradient: (a) *Rhodococcus equi* C7; (b) *Rhodococcus rhodochrous* N54.

Fig. 2. Denaturation curves of duplexes between 23S ^{14}C -labelled rRNA from *Rhodococcus equi* C7 and filter-bound DNA from other *Rhodococcus* strains and *E. coli*.

Fig. 3. Similarity map of duplexes between 23S ^{14}C -labelled rRNA from *Rhodococcus rhodochrous* N54 and DNA from representative strains of *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Saccharopolyspora* and *Streptomyces*. $T_{m(e)}$ and 'percentage of rRNA binding' are defined in Methods. Strains belonging to genera recognized on the basis of phenotypic properties are surrounded by a closed line. The lines do not represent sharp generic boundaries as the shape and dimensions of the defined taxa are limited by the numbers of strains examined.

plotting, for each reference system, $T_{m(e)}$ against percentage of rRNA binding (Figs 3 and 4).

In rRNA similarity maps each strain has a fixed position. It can be seen from Figs 3 and 4 that while all of the test strains form stable duplexes they can be divided into four groups when the amount of binding is also taken into account. The groups contain the rhodococci, mycobacteria, nocardiae and streptomycetes, respectively. The position of *Saccharopolyspora hirsuta* N745 is equivocal as it clusters with the streptomycetes in one reference system (Fig. 4) but does not fall into any of the four groups in the other reference system (Fig. 3).

Fig. 4. Similarity map of duplexes between 23S ^{14}C -labelled rRNA from *Rhodococcus equi* C7 and DNA from representative strains of *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Saccharopolyspora* and *Streptomyces*. For further details, see legend to Fig. 3.

DISCUSSION

The results of the present nucleic acid pairing experiments have made it possible to establish the phylogenetic relationships between actinomycetes that show little genetic similarity in DNA:DNA pairing assays (Bradley & Mordarski, 1976; Mordarski *et al.*, 1976, 1977, 1978, 1980). These preliminary data, therefore, provide yet further evidence that the segment of the bacterial genome coding for rRNA is more conserved in the course of evolution than the bulk of the genome (Doi & Igarashi, 1965; Dubnau *et al.*, 1965) and strongly suggest that a close phylogenetic relationship exists between *Rhodococcus*, *Mycobacterium*, *Nocardia*, *Saccharopolyspora* and *Streptomyces*. Thus, all of the representatives of these taxa formed stable duplexes in both the *R. equi* and *R. rhodochrous* reference systems. Additional comparative studies using representatives of other taxa are, however, needed to determine whether the actinomycetes form a natural group. The base composition data help to confirm that the actinomycetes are rich in guanine and cytosine (Mordarski *et al.*, 1978).

It was encouraging that the representatives of the genera *Rhodococcus*, *Mycobacterium*, *Nocardia* and *Streptomyces* occupied recognizable areas on the similarity maps. Nevertheless, additional representative strains should be examined to confirm the position of these taxa on similarity maps and to establish the shape and size of the area occupied by each of the genera. It will also be interesting to see if mycolic acid-containing actinomycetes with a wall chemotype IV have a closer affinity with one another than with related actinomycetes which lack mycolic acids (Goodfellow & Minnikin, 1977, 1978; Minnikin & Goodfellow, 1980). The preliminary nature of these data, however, makes it premature to try to correlate rRNA similarities with phenotypic properties, though there is some evidence that such comparisons may provide a basis for selecting phenotypically conserved characters which represent cistrons that are partially conserved (De Smedt & De Ley, 1977; Johnson & Francis, 1975).

Estimates of rRNA similarities can also be of value in determining the relationships of misclassified or poorly classified strains (De Smedt & De Ley, 1977). Such studies should,

therefore, be of value in establishing the status of strains such as *Arthrobacter variabilis*, *Corynebacterium equi*, *C. hoagi* and *C. hydrocarboclastus* which have many phenotypic characters in common with representatives of *Corynebacterium sensu stricto* and *Rhodococcus* (Minnikin *et al.*, 1978; Minnikin & Goodfellow, 1980). The proposal to transfer *C. equi* to the genus *Rhodococcus* (Goodfellow & Alderson, 1977) is not contradicted by the rRNA similarity data and is supported both by the present and past base composition studies (Yamada & Komagata, 1970). Thus, the base ratio estimations for *R. equi* C7 and N829 are within the 65.1 to 72.7 mol % GC range expected for rhodococci using the thermal denaturation method (Mordarski *et al.*, 1980).

The base composition and rRNA : DNA similarity data support the integrity of the genus *Rhodococcus* and its separation from related mycolic acid-containing actinomycetes classified in the genera *Mycobacterium* and *Nocardia*. Further DNA : rRNA pairing experiments are, however, required to confirm these preliminary observations and to determine the relationships between true corynebacteria and rhodococci.

REFERENCES

- BRADLEY, S. G. & MORDARSKI, M. (1976). Association of polydeoxyribonucleotides of deoxyribonucleic acids from nocardioform bacteria. In *The Biology of the Nocardiae*, pp. 310–336. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.
- BURTON, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochemical Journal* **62**, 315–323.
- CROMBACH, W. H. J. (1978). DNA base ratios and DNA hybridisation studies of coryneform bacteria, mycobacteria and nocardiae. In *Coryneform Bacteria*, pp. 161–179. Edited by I. J. Bousfield & A. G. Calley. London: Academic Press.
- DE LEY, J. & DE SMEDT, J. (1975). Improvements of the membrane filter method for DNA : rRNA hybridization. *Antonie van Leeuwenhoek* **41**, 287–307.
- DE SMEDT, J. & DE LEY, J. (1977). Intra- and intergeneric similarities of *Agrobacterium* ribosomal ribonucleic acid cistrons. *International Journal of Systematic Bacteriology* **27**, 222–240.
- DOI, R. H. & IGARASHI, R. T. (1965). Conservation of ribosomal and messenger ribonucleic acid cistrons in *Bacillus* species. *Journal of Bacteriology* **90**, 384–390.
- DUBNAU, D., SMITH, I., MORELL, P. & MARMUR, J. (1965). Gene conservation in *Bacillus* species. I. Conserved genetic and nucleic acid sequence homologies. *Proceedings of the National Academy of Sciences of the United States of America* **54**, 491–498.
- GOODFELLOW, M. (1971). Numerical taxonomy of some nocardioform bacteria. *Journal of General Microbiology* **69**, 33–80.
- GOODFELLOW, M. & ALDERSON, G. (1977). The actinomycete genus *Rhodococcus*: a home for the 'rhodochrous' complex. *Journal of General Microbiology* **100**, 99–122.
- GOODFELLOW, M. & MINNIKIN, D. E. (1977). Nocardioform bacteria. *Annual Review of Microbiology* **31**, 159–180.
- GOODFELLOW, M. & MINNIKIN, D. E. (1978). Numerical and chemical methods in the classification of *Nocardia* and related taxa. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I)*, Supplement **6**, 43–51.
- GOODFELLOW, M., ORLEAN, P. A. B., COLLINS, M. D., ALSHAMAONY, L. & MINNIKIN, D. E. (1978). Chemical and numerical taxonomy of strains received as *Gordona aurantiaca*. *Journal of General Microbiology* **109**, 57–68.
- JOHNSON, J. L. & FRANCIS, B. S. (1975). Taxonomy of the clostridia: ribosomal ribonucleic acid homologies among the species. *Journal of General Microbiology* **88**, 229–244.
- JOHNSON, J. L., ANDERSON, R. S. & ORDAL, E. J. (1970). Nucleic acid homologies among oxidase-negative *Moraxella* species. *Journal of Bacteriology* **101**, 568–573.
- JONES, D. (1975). A numerical taxonomic study of coryneform and related bacteria. *Journal of General Microbiology* **87**, 52–96.
- JONES, D. (1978). An evaluation of the contributions of numerical taxonomic studies to the classification of coryneform bacteria. In *Coryneform Bacteria*, pp. 13–46. Edited by I. J. Bousfield & A. G. Calley. London: Academic Press.
- KEDDIE, R. M. & CURE, G. L. (1978). Cell wall composition of coryneform bacteria. In *Coryneform Bacteria*, pp. 47–83. Edited by I. J. Bousfield & A. G. Calley. London: Academic Press.
- LECHEVALIER, M. P. & LECHEVALIER, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *International Journal of Systematic Bacteriology* **20**, 435–444.
- MC CONKEY, E. H. (1967). The fractionation of RNA's by sucrose gradient centrifugation. *Methods in Enzymology* **12**, 620–634.
- MARMUR, J. & DOTY, P. (1962). Determination of base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.
- MEYS, W. H. & SCHILPEROORT, R. A. (1971). Determination of the amount of DNA on nitrocellulose membrane filters. *FEBS Letters* **12**, 166–168.

- MINNIKIN, D. E. & GOODFELLOW, M. (1976). Lipid composition in the classification and identification of nocardiae and related taxa. In *The Biology of the Nocardiae*, pp. 160–219. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.
- MINNIKIN, D. E. & GOODFELLOW, M. (1980). Lipid composition in the classification and identification of acid fast bacteria. In *Microbiological Classification and Identification*, pp. 189–256. Edited by M. Goodfellow & R. G. Board. London: Academic Press.
- MINNIKIN, D. E., GOODFELLOW, M. & COLLINS, M. D. (1978). Lipid composition in the classification and identification of coryneform and related taxa. In *Coryneform Bacteria*, pp. 85–160. Edited by I. J. Bousfield & A. G. Calley. London: Academic Press.
- MOORE, R. L. & MCCARTHY, B. J. (1967). Comparative study of ribosomal ribonucleic acid cistrons in enterobacteria and myxobacteria. *Journal of Bacteriology* **94**, 1066–1074.
- MOORE, R. L. & MCCARTHY, B. J. (1969). Base sequence homology and renaturation studies of the deoxyribonucleic acid of extremely halophilic bacteria. *Journal of Bacteriology* **99**, 255–262.
- MORDARSKI, M., SZYBA, K., PULVERER, G. & GOODFELLOW, M. (1976). Deoxyribonucleic acid reassociation in the classification of the 'rhodochrous' complex and allied taxa. *Journal of General Microbiology* **94**, 235–245.
- MORDARSKI, M., GOODFELLOW, M., SZYBA, K., PULVERER, G. & TKACZ, A. (1977). Classification of the 'rhodochrous' complex and allied taxa based upon deoxyribonucleic acid reassociation. *International Journal of Systematic Bacteriology* **27**, 31–37.
- MORDARSKI, M., GOODFELLOW, M., SZYBA, K., PULVERER, G. & TKACZ, A. (1978). Deoxyribonucleic acid base composition and homology studies on *Rhodococcus* and allied taxa. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I)*, Supplement **6**, 99–106.
- MORDARSKI, M., GOODFELLOW, M., SZYBA, K., TKACZ, A., PULVERER, G. & SCHAAL, K. P. (1980). Deoxyribonucleic acid reassociation in the classification of the genus *Rhodococcus*. *International Journal of Systematic Bacteriology* (in the Press).
- PACE, B. & CAMPBELL, L. L. (1971*a*). Homology of ribosomal ribonucleic acid of *Desulfovibrio* species with *Desulfovibrio vulgaris*. *Journal of Bacteriology* **106**, 717–719.
- PACE, B. & CAMPBELL, L. L. (1971*b*). Homology of ribosomal ribonucleic acid of diverse bacterial species with *Escherichia coli* and *Bacillus stearothermophilus*. *Journal of Bacteriology* **107**, 543–547.
- PALLERONI, N. J., KUNISAWA, R., CONTOPOULOU, R. & DOUDOROFF, M. (1973). Nucleic acid homologies in the genus *Pseudomonas*. *International Journal of Systematic Bacteriology* **23**, 333–339.
- ROWBOTHAM, T. J. & CROSS, T. (1977). *Rhodococcus coprophilus* sp. nov.: an aerobic nocardioform actinomycete belonging to the 'rhodochrous' complex. *Journal of General Microbiology* **100**, 123–138.
- SAITO, H. & MIURA, K. I. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochimica et biophysica acta* **72**, 619–629.
- SHIRLING, E. B. & GOTTLIEB, D. (1968). Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. *International Journal of Systematic Bacteriology* **18**, 69–189.
- SHIRLING, E. B. & GOTTLIEB, D. (1972). Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. *International Journal of Systematic Bacteriology* **22**, 265–394.
- TAKAHASHI, H., SAITO, H. & IKEDA, Y. (1967). Species specificity of the ribosomal RNA cistrons in bacteria. *Biochimica et biophysica acta* **134**, 124–133.
- YAMADA, K. & KOMAGATA, K. (1970). Taxonomic studies on coryneform bacteria. III. DNA base composition of coryneform bacteria. *Journal of General and Applied Microbiology* **16**, 215–224.
- ZOPF, W. (1891). Über Ausscheidung von Fettfarbstoffen (Lipochromen) seitens gewisser Spaltpilze. *Berichte der Deutschen botanischen Gesellschaft* **9**, 22–28.