Characterization of Deoxyribonucleic Acids from Streptomycetes and Nocardiae

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The relationships among selected streptomycetes, nocardiae, and mycobacteria have been determined, based upon the base composition of their deoxyribonucleic acid (DNA) and upon the ability of their denatured DNA to anneal with singlestranded reference DNA. The streptomycetes constituted a homogeneous group whose DNA contained between 69 and 73 mole % guanine + cytosine (% GC). Moreover, the streptomycetes examined showed 37 to 88% homology with the Streptomyces venezuelae and S. rimosus reference DNA. The nocardial and mycobacterial DNA both contained 62 to 69% GC. The nocardial strains studied fell into either a 62 to 64% GC group or a 68 to 69% GC group, indicating that they should not be assigned to a single species. The nocardiae having 68 to 69% GC showed 24 to 44% homology with S. venezuelae reference DNA. In competition experiments, wherein unlabeled heterologous DNA interfered with binding of labeled homologous DNA, the nocardial DNA with 68 to 69% GC showed a greater degree of homology with the streptomycetes than did the nocardial DNA with 62 to 64% GC. In addition, the DNA from spores of S. venezuelae was cursorily examined, and interactions between S. venezuelae denatured DNA and polyribonucleotides were sought. The buoyant density of the DNA from S. venezuelae spores was distinctly less than that from mycelia. Moreover, denatured S. venezuelae DNA formed a dense complex with polyriboguanylate.

In systematic studies, only a small portion of an organism's overt characteristics have generally been considered. Although reasonably useful taxonomic systems have been devised upon the bases of a limited number of cardinal features, on the one hand, and of overall similarity with respect to multitudes of unselected characteristics, on the other hand, similarity between the genetic determinants themselves seems the most appropriate criterion for microbial classification (1). The deoxyribonucleic acid (DNA) base composition provides helpful but limited systematic information. Two organisms having the same DNA composition may be, but are not necessarily, closely related (14). For definitive studies on biological relatedness, genetic homol-ogy per se must be determined. Two organisms possess extensive genetic homology if they are able to undergo syncytic recombination. However, a limited number of genetic determinants can be transferred from one organism to another by an autonomous episome whose DNA composition may differ appreciably from that of the recipient (6). Because syncytic recombinational studies are technically tedious and are biologically restricted by factors such as compatibility sys-

tems (3), the extent of genetic homology can better be assessed by measuring renaturation between single-stranded DNA preparations of diverse origin (12).

In the present study, the relationships among selected streptomycetes, nocardiae, and mycobacteria were determined, based upon the base composition of their DNA and upon the ability of their denatured DNA to anneal with singlestranded reference DNA. Concurrently, the DNA from the spores of Streptomyces venezuelae was cursorily examined, and interactions between S. venezuelae denatured DNA and polyribonucleotides were sought. Our results demonstrate a continuous spectrum of genetic homology among members of the genera Streptomyces and Nocardia. In addition, we found that the DNA from S. venezuelae spores has properties different from those of mycelial DNA and that denatured S. venezuelae DNA interacts with polyriboguanylate.

MATERIALS AND METHODS

The organisms used in this study were primarily members of the genera *Streptomyces* and *Nocardia*. The culture designations and their sources are given in Table 1. The stock streptomycete cultures were

TABLE 1. Principal stocks

^a N. M. McClung, University of South Florida, Tampa; E. McCoy, University of Wisconsin, Madison; J. B. Clark, University of Oklahoma, Norman; J. N. Adams, University of South Dakota, Vermillion; S. A. Waksman, Rutgers, The State University, New Brunswick, N.J.; J. D. Stout, Soil Bureau, DSIR, Wellington, New Zealand; ATCC, American Type Culture Collection, Rockville, Md.; IMRU, Institute of Microbiology, Rutgers, The State University, New Brunswick, N.J.; NRRL, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill.

propagated on tomato paste-oatmeal-agar medium (13). The stock cultures of the other actinomycetes, mostly nocardiae, were propagated on peptone-yeast extract-agar medium (15). All streptomycete stock cultures were incubated at 30 C for 7 to 14 days, whereas nocardial stock cultures were incubated at 30 C for 3 to 7 days. Mycelia for DNA extraction were grown in peptone-yeast extract broth shaken at 30 C until the absorbancy of the culture reached 0.6 to 0.8 (as measured at 620 mµ with a Bausch & Lomb Spectronic-20 colorimeter). The mycelia were harvested by centrifugation, washed thrice with salineethylenediaminetetraacetic acid (EDTA) solution (19), and frozen at -20 C until needed. The streptomycete hyphae were lysed by a combination of lysozyme, Pronase, and sodium dodecyl sulfate (SDS). To lyse nocardial hyphae by this combination of treatments, the cell mass had to be previously extracted with acetone and diethyl ether. After lysis, DNA was isolated by the Marmur procedure (19).

To extract DNA from mycobacteria and from streptomycete spores, the cells had to be ruptured mechanically. S. venezuelae spores, for example, were lyophilized; 0.5 g of lyophilized spores were mixed with 1 g of acid-washed glass beads $(20 \ \mu$ in diameter; 3 M Co., Minneapolis, Minn.) in a mortar and ground manually for 5 min. Pulverized dry ice was added during grinding. The ground spores were suspended

in saline-EDTA; the suspension was digested with Pronase and treated with SDS. The DNA was isolated as described above.

The buoyant density of each isolated DNA was determined by the technique of Meselson et al. (20), with a Spinco (model E) analytical ultracentrifuge operated at 44,770 rev/min at 20 C for 18 hr. The resulting ultraviolet absorption films were traced with a microdensitometer (Beckman model RB Analytrol film densitometer, slit width of 100 μ). The buoyant density of each unknown DNA was then calculated by use of the position of an internal standard of *Escherichia coli* DNA as a reference. The buoyant density of *E. coli* DNA was taken to be 1.710 g/cc (21).

The degree of genetic homology between streptomycete and nocardial DNA preparations was assessed by measuring the extent of in vitro hybrid formation between single-stranded DNA molecules of different origin; the agar-gel technique developed by McCarthy and Bolton (18) was used. S. venezuelae S13 DNA and S. rimosus DNA were labeled with uracil-2-14C in a medium composed of glucose, 20 g; peptone, 5 g; $K_2HPO_4 \cdot 3H_2O$, 5 g; NH_4NO_3 , 2 g; asparagine, 1 g; MgSO4 7H2O, 0.5 g; and deionized water, 1 liter. The specific activity of the labeled S. venezuelae DNA was 2,400 counts per min per µg and that of S. rimosus was 1,600 counts per min per µg. The unlabeled DNA preparations were adjusted to a concentration of 1 mg/ml of 0.1 saline-sodium citrate (SSC), and 5-ml samples were heated in a boilingwater bath for 7 min. These heat-denatured DNA preparations were embedded in agar to give concentrations of 150 to 250 μ g of DNA/g of wet agar. Reannealing was achieved by incubating 0.5 g of wet DNA-agar containing 10 parts of immobilized DNA with 0.75 ml of 2 SSC containing 1 part of labeled DNA at 60 C for 15 hr. Although a ratio of 100 parts of immobilized DNA to 1 part of labeled DNA gave more binding, control experiments established that a ratio of 10:1 gave more reproducible, specific retention of the mobile DNA. The unbound ¹⁴C-labeled DNA was removed by 10 washes with 15 ral of double-strength SSC at 60 C; the hybridized DNA was then removed by 5 washes with 0.01 SSC at 75 C. The eluted DNA, with added carrier (thymus DNA), was precipitated with 5% trichloroacetic acid, trapped on membrane filters, dried, and radioassayed. Samples were counted with a Tracerlab FD-1 gas-flow counter with an ultrathin window.

RESULTS

Gross analyses. The buoyant densities of the actinomycete DNA preparations studied ranged from 1.7211 to 1.7313 g/cc. The buoyant density of the heat-denatured DNA was about 0.01 g/cc greater than that of the corresponding native DNA (Table 2). All DNA preparations, whether native or heat-denatured, showed only a single, symmetrical band in the cesium chloride density gradient. The mole % guanine plus cytosine (% GC) of the DNA was calculated according to the

	Buoyant		
Organism	Dena- tured	Native	% GC
Streptomyces coelicolor			
Müller	NDª	1.7313	72.8
S. aureofaciens	1.7407	1.7310	72.4
S. coelicolor 1945	ND	1.7307	72.1
S. cinnamomeus	ND	1.7307	72.1
S. albus	ND	1.7306	72.0
S. fradiae	ND	1.7304	71.8
S. violaceoruber 199	1.7413	1.7303	71.8
S. violaceoruber 16	ND	1.7301	71.5
S. venezuelae 13	1.7405	1.7300	71.4
S. venezuelae 86	ND	1.7298	71.2
S. rimosus	1.7413	1.7290	70.4
S. griseus		1.7290	70.4
S. erythraeus	ND	1.7284	69.8
Actinophage MSP2	1.7383	1.7270	68.4
Nocardia corallina S5		1.7273	68.7
N. corallina 78	1.7390	1.7272	68.6
N. opaca 76	1.7372	1.7267	68.1
N. canicruria 1574	1.7351	1.7223	63.6
N. opaca 765A	1.7346	1.7222	63.5
N. canicruria 57		1.7214	62.7
N. erythropolis	1.7335	1.7212	62.5
N. corallina 305	1.7326	1.7211	62.3
Mycobacterium phlei	ND	1.7275	68.9
M. kansasii.	ND	1.7271	68.5
M. tuberculosis	ND	1.7236	64.9

 TABLE 2. Buoyant density and base composition of actinomycete DNA preparations

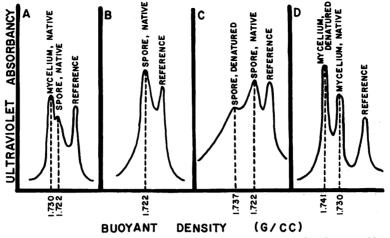
^a Not determined.

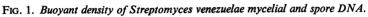
equation, d = 1.660 + 0.098 (% GC), where d = buoyant density (21). The calculated % GC of the streptomycete DNA preparations ranged between 69.8 and 72.8, those of nocardiae were between 62.3 and 68.7, and those of mycobacteria were between 64.9 and 68.9. The genetically recombinable pairs, *S. violaceoruber* 199 by 16 and *N. canicruria* by *N. erythropolis*, have essentially the same DNA base compositions (71.4 to 71.5% GC and 62.5 to 63.6% GC, respectively). Some cultures bearing the same species designations had distinctly different base compositions (for example, strains of *N. corallina* and *N. opaca*).

The buoyant density of the DNA from spores of *S. venezuelae* S13 was found to be 1.7222 g/cc.The buoyant density of DNA from the mycelium of *S. venezuelae* S13, isolated under conditions identical to those used for spore DNA had a buoyant density of 1.7300 g/cc. This is the same value found for DNA from mycelia lysed with lysozyme, Pronase, and SDS. When the spore DNA was heat-denatured, it formed a single band with a density of 1.7371 g/cc, indicating that the isolated spore DNA was double-stranded (Fig. 1).

To detect interactions between polyribonucleotides and denatured S. venezuelae S13 DNA, 20 μ g of polyguanylate (poly G), polyuridylate (poly U), polycytidylate (poly C), polyadenylate (poly A), or polyinosinate (poly I) was mixed with 1 μg of native DNA, and the DNA in the mixture was heat-denatured. (All polyribonucleotides were obtained from Miles Chemical Co., Elkhart, Ind.) After cooling, CsCl was added, followed by addition of the E. coli reference DNA (16). The position of the ultraviolet-absorbing band in the cesium chloride gradient formed by denatured S. venezuelae DNA in mixture with poly U or poly A was the same as that of the control denatured DNA. With poly C and poly I, an increase in the amount of DNA in the band corresponding to native DNA was observed. It should be noted that the poly C preparation was substantially degraded, and it did not give a sharp boundary at the bottom of the gradient. In the presence of poly G, the band corresponding to denatured DNA was essentially missing from the gradient. and only a small amount of material having the buoyant density of native DNA was observed. The remainder of the denatured DNA, plus the added poly G, sedimented to the bottom of the gradient (Fig. 2). When the concentration of the added poly G was reduced 100-fold (0.2 $\mu g/\mu g$ of DNA), the poly G-denatured DNA complex appeared in a single broad band within the gradient at a position corresponding to a density higher than that of the control denatured DNA (Fig. 3).

DNA homologies. 14C-labeled, sheared, denatured S. venezuelae S13 DNA or S. rimosus DNA was hybridized with DNA from 12 streptomycete cultures and DNA from actinophage MSP2, embedded in agar. The data are expressed as the relative amount of DNA bound by a heterologous system compared to that bound by the homologous system. In the homologous S. venezuelae system, 40% of the labeled DNA was bound; in the S. rimosus system, 34% of the labeled DNA was bound. The relative amount of labeled DNA bound by the heterologous streptomycete systems ranged between 37 and 88% of that by the homologous system. S. griseus, S. venezuelae 86, and S. rimosus DNA preparations showed the largest degree of homology with S. venezuelae S13 DNA. Similarly, S. griseus and S. venezuelae S13 showed the greatest binding of S. rimosus sheared DNA. S. fradiae DNA showed only 38% homology with either S. venezuelae or S. rimosus. A significant difference was noted in the ability of S. violaceoruber S199, compared with S. violaceoruber S16, to bind S. venezuelae DNA. This difference in binding capability of the two S. violaceoruber DNA preparations was much less when S. rimosus





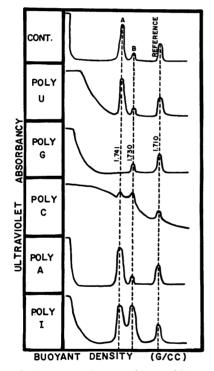


FIG. 2. Interactions between denatured Streptomyces venezuelae DNA and synthetic polyribonucleotides. (A) Heat-denatured DNA band; (B) native DNA band.

was the source of the reference DNA. Actinophage MSP2 DNA showed only 11% homology with the DNA of its host, *S. venezuelae*; *E. coli*, *Myxococcus xanthus*, and salmon sperm DNA bound less than 5% of the streptomycete DNA (Table 3). Accordingly, any binding in excess of

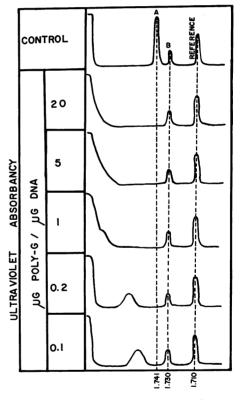




FIG. 3. Interactions between denatured Streptomyces venezuelae DNA and polyriboguanylate (poly-G). (A) Heat-denatured DNA band; (B) native DNA band.

5% of the homologous binding represented specific binding rather than a nonspecific interaction between two DNA strands rich in guanine and cytosine. An appreciable part of the 11%

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TABLE	3.	Homology	of	DNA	of	selected
st	reptor	nycetes with	that	of Stre	ptom	yces
		venezuelae o	or S.	rimosu	5	

Test organism	Relative amount of DNA bound by reference DNA			
	S. venezuelae	S. rimosus		
Streptomyces venezuelae S13 S. griseus S. venezuelae S. rimosus S. aureofaciens S. violaceoruber S. coelicolor Müller S. coelicolor Müller S. fradiae S. violaceoruber S. dibus S. coelicolor Müller S. fradiae S. violaceoruber S. stolaceoruber S. stolaceoruber S. stolaceoruber S. violaceoruber S. stolaceoruber S. st	$ \begin{array}{c} 100\\ 88 \pm 5\\ 79 \pm 5\\ 78 \pm 4\\ 64 \pm 4\\ 58 \pm 6\\ 54 \pm 6\\ 52 \pm 6\\ 51 \pm 6\\ 48 \pm 6\\ 37 \pm 6\\ 11 \pm 2\\ 5 \pm 2\\ 4 \pm 1\\ 2 \pm 1\\ \end{array} $	$77 \pm 4 \\ 72 \pm 6 \\ 62 \pm 6 \\ 100 \\ 63 \pm 6 \\ 56 \pm 6 \\ 57 \pm 6 \\ 42 \pm 6 \\ 51 \pm 6 \\ 68 \pm 7 \\ 48 \pm 7 \\ 48 \pm 7 \\ 48 \pm 6 \\ ND^a \\ 3 \pm 2 \\ ND \\ 4 \pm 2 \\ 100 \\ $		
Blank agar	0	0		

^a Not determined.

binding of *S. venezuelae* DNA to phage MSP2 DNA, therefore, is specific.

DNA-agar preparations were also prepared, by use of DNA from *N. opaca* 76, *N. corallina* 78, and *N. corallina* S5. These were tested for extent of hybridization with an *S. venezuelae* S13 reference DNA. In this series, the homologous system bound only 25% of the labeled reference DNA. The three *Nocardia* strains showed significant homology with *S. venezuelae* (Table 4).

Base sequence complementarity among DNA molecules can also be studied by means of competition reactions (12). Accordingly, similarity is expressed in terms of the extent of interference by unlabeled heterologous DNA with the binding of the labeled homologous DNA. In our competition experiments, S. venezuelae S13 was used as the homologous system and seven heterologous nocardial DNA preparations were tested for ability to interfere competitively with homologous binding. The seven nocardial preparations fell into two groups. The DNA from N. corallina 305, N. canicruria, N. opaca 765A, and N. erythropolis showed less competition than did that from the other three. E. coli DNA showed practically no competition with S. venezuelae DNA, whereas unlabeled homologous S. venezuelae DNA showed the greatest competition (Fig. 4).

 TABLE 4. Homology of DNA of three Nocardia strains with that of Streptomyces venezuelae

Test organism	Relative amount of DNA bound by S. venezuelae reference DNA
N. opaca 76	44 ± 4
N. corallina 78	37 ± 4
N. corallina S5	24 ± 4

DISCUSSION

The confused state of the taxonomy of the group of organisms designated as N. corallina and N. opaca is well illustrated by this study. Cultures bearing the same epithet are clearly dissimilar, yet Gordon and Mihm (9, 10) reduced N. corallina 78 and N. opaca 765A to synonymy with Mycobacterium rhodochrous. We consider N. corallina 78, N. corallina S5, and N. opaca 76 to be distinctly different from N. canicruria, N. opaca 765A, N. erythropolis, and N. corallina 305, based upon the present results and upon phagetyping data (4). We concur with Gordon (8) that the choice of the correct generic assignment is especially difficult; arguments may be presented supporting assignment to Corynebacterium, Mycobacterium, or Nocardia. These organisms do show appreciable homology with streptomycetes; therefore, we presently favor retaining the oldest generic designation (Nocardia), which also suggests meaningful relationships with the streptomycetes.

Our results with streptomycetes largely confirm previous findings (2). The % GC values, based upon buoyant density, agree well with those obtained earlier by thermal denaturation. In contrast, Frontali et al. (7) reported that % GC based upon thermal denaturation was about 5%higher than that based upon buoyant density. It must be kept in mind that the relationship of %GC to temperature of melting is not linear at either end of the curve, and that extrapolation based upon references in the middle of the curve will result in substantial errors. Both the gross DNA analyses and the homology studies justify the separation of S. coelicolor from S. griseus and the renaming of the S. violaceoruber cultures which have been mislabeled as S. coelicolor (17). Based upon our data, and the compilation of Hill (11), we suggest that the gross DNA base compositions of the members of a genus will not vary by more than 10%.

The recombinable pairs, S. violaceoruber 199 by 16 and N. canicruria by N. erythropolis, have similar DNA compositions (about 72 and 63 %, respectively). Moreover, the homology results

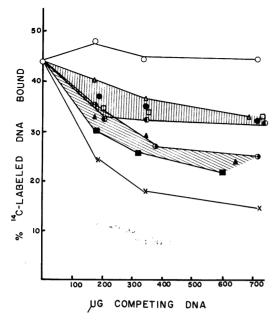


FIG. 4. Inhibition of homologous hybridization by nocardial DNA. The immobile phase consisted of 330 μg of Streptomyces venezuelae DNA/agar column. The mobile phase contained 3.3 μg of ¹⁴C-labeled S. venezuelae DNA (2,400 counts per min per μg of DNA) and 0 to 700 μg of unlabeled nocardial DNA. (\triangle) Nocardia corallina N305. (\bigcirc) N. canicruria 1574. (\Box) N. opaca 765A. (\bigcirc) N. erythropolis. (\bigcirc) N. corallina S5. (\triangle) N. corallina 78. (\bigcirc) N. opaca 76. (\times) S. venezuelae. (\bigcirc) Escherichia coli.

place the two nocardias in the same group. With S. violaceoruber, however, S. venezuelae DNA reacts differently with the DNA from the two strains. With S. rimosus DNA as the reference, the two S. violaceoruber strains react similarly. These results suggest that these two strains, although recombinable, have appreciable differences in DNA base sequence. Studies examining the homologies among many different S. violaceoruber strains are in progress. It should be noted that many factors affected the extent and specificity of the binding of labeled DNA to the DNA-agar in our experiment. The amount of reannealing decreased as the ratio of the total liquid volume to the volume of the agar-gel increased. However, a ratio of 2.5:1 was found to be the minimum value that assured uniform penetration of the liquid phase into the particles of agar. Moreover, the amount of DNA trapped in the agar was an important variable. The percentage of labeled DNA bound decreased with decreasing DNA content of the agar. In addition, the extent of reannealing increased as the ratio of

the amount of sheared, labeled DNA to the amount of DNA in the agar was reduced. Although 60% of the presented labeled DNA was bound when a ratio of labeled to agar-trapped DNA of 0.01:1 was used, a ratio of 0.1:1 gave more reproducible results with approximately 40% binding of the labeled DNA. Finally, the extent of washing had to be sufficient to remove essentially all of the unbound labeled DNA without appreciable leaching of the reannealed hybrid DNA from the agar. Previous experiments had shown that 10 washes with 15 ml of 2 SSC at 60 C satisfactorily eluted denatured DNA without loss of hybrid DNA (Tewfik et al., in press). An important variable, not examined by us, was the incubation temperature for renaturation. In general, the incubation temperature should be 25 C lower than the "melting points" of the DNA preparations examined. For actinomycete DNA, this would be about 70 C (95 C - 25 C). Our use of a lower incubation temperature (60 C) might raise some question about the specificity of the binding percentages reported here. In our study, however, the retention of labeled, sheared DNA by the DNA-agar was clearly not attributable to rather nonspecific interactions among denatured DNA species having high GC levels. The DNA of M. xanthus, which contains 70% GC (2), was retained no more extensively than was E. coli DNA, which contains 50% GC.

During studies on the buoyant densities of actinomycete DNA preparations, DNA from S. venezuelae S13 spores was included. The DNA of the spore displays a buoyant density appreciably less than that of the mycelial DNA. The DNA is double-stranded, based upon the shift in density induced by thermal denaturation. The denatured spore DNA possesses a buoyant density less than that of denatured mycelial DNA. A similar discrepancy in buoyant density of spore and vegetative DNA has been observed with Bacillus cereus, but its spore DNA is more dense than the vegetative DNA (5). Bednar and Frea (Bacteriol. Proc., p. 173, 1967) sought, but did not find, differences in the buoyant density of DNA from spores and mycelium of S. fradiae. The cause for the lightness of streptomycete spore DNA has not been established, but it is presumably attributable to a change in secondary structure or to binding with some other cellular component and not to an actual change in base composition. The change in the buoyant density characteristics of the spore DNA is a property of the individual strands rather than of the duplex. In buoyant density equilibrium centrifugation, no satellite DNA

bands were observed with either native or denatured DNA preparations. This indicates that there are no episomes with DNA base compositions substantially different from those of the actinomycete nucleoid DNA. The denatured DNA of *S. venezuelae* does interact with polyriboguanylate to form a dense complex. The two strands of the denatured DNA seemed to react equally with the poly G; poly I and poly C seem to retard denaturation or facilitate renaturation of the denatured DNA.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-07733 from the National Institute of Allergy and Infectious Diseases. The senior author was the recipient of a scholarship from the Institute of Education, United Arab Republic.

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