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Genetic Differentiation by Nucleic Acid Homology

I. Relationships Among *Mycoplasma* Species of Man

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ABSTRACT

REICH, PAUL R. (National Institutes of Health, Bethesda, Md.), NORMAN L. SOMERSON, CAROL J. HYBNER, ROBERT M. CHANOCK, AND SHERMAN M. WEISSMAN. Genetic differentiation by nucleic acid homology. I. Relationships among *Mycoplasma* species of man. J. Bacteriol. **92**:302-310. 1966.—Genetic relatedness among human mycoplasmas was evaluated by measuring the amount of nucleic acid hybrid retained on a membrane filter. Hybrids were formed from deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) derived from representative strains of seven serologically distinct human *Mycoplasma* species. The results indicate that serologically distinct human *Mycoplasma* species can also be distinguished by the homology techniques. Low-level cross-reactivity was observed among nucleic acids derived from the seven species. Genetic heterogeneity was demonstrated among three strains of *M. salivarium* and between two strains of *M. orale* type 2. In contrast, comparison of three strains and three passage levels of *M. pneumoniae* revealed them to be indistinguishable. *M. pneumoniae* appears to be the most distinct of all human mycoplasmas, as shown by both homology and the high buoyant density value of its DNA. Nucleic acids from mycoplasmas which had identical buoyant densities were in some cases differentiable. Mycoplasmas with different DNA buoyant densities were invariably distinguishable by the homology technique.

Nucleic acid homology techniques have been used by several investigators to study the relationship between mycoplasmas and bacteria. These methods depend upon the ability of single-stranded nucleic acids obtained from genetically related organisms to form hybrids. For example, use of an agar column method that measures the amount of hybrid formation between two strands of deoxyribonucleic acid (DNA) has provided evidence that two avian mycoplasmas are not derived from *Haemophilus gallinarum* and also that *Mycoplasma pneumoniae* is not a stable L form of *Streptococcus* MG (12, 20). In other investigations, a membrane filter method for measuring DNA-RNA (deoxyribonucleic acid-ribonucleic acid) hybrid formation was used to study genetic relatedness among mycoplasmas (19). A *M. hominis* type 1 isolate was easily distinguished by this technique from strains belonging to other human *Mycoplasma* species. Four different *M. hominis* type 1 isolates, although serologically closely related, were differentiable from each other.

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The present report describes the application of this DNA-RNA homology method to a study of six additional, serologically distinct, human *Mycoplasma* species. Three species—*M. orale* type 2, *M. salivarium*, and *M. pneumoniae*—were investigated for evidence of genetic heterogeneity. The data from these nucleic acid homology studies are compared with the results of the corresponding DNA buoyant density studies.

MATERIALS AND METHODS

Organisms. The source of each prototype human *Mycoplasma* strain and references to their isolation and identification are listed in Table 1. Strains of *M. salivarium* were obtained from throats of patients at Children's Hospital, Washington, D.C. All mycoplasmas were subcultured on agar and in broth media. Except for the *M. salivarium* isolates and *M. pneumoniae* strain FV-3-63, all strains had been cloned by a technique described elsewhere (25). Growth-inhibition testing (6) confirmed the serological identification of all *Mycoplasma* strains.

M. laidlawii PG9 (11) was obtained from D.G. ff. Edward, and the avian species, *M. gallisepticum* S6 (29), was received from Dr. Madden.

TABLE 1. *Source of human Mycoplasma strains*

Serological type or species	Strain	Recovered from	Obtained from (reference)
M. hominis type 1	PG21	Genital tract	Edward (17)
	Botteicher	Oropharynx	Mufson (28)
	V2785	Oropharynx	Mufson (28)
	DC63	Oropharynx	Mufson (15)
M. hominis type 2	Campo, PG27 (*M. arthritidis*)	Genital tract	Edward, originally from Dienes; human origin questionable (9)
M. fermentans	G, PG18	Genital tract	Edward (22)
M. orale type 1	CH19299	Oropharynx	Canchola (25)
M. orale type 2	DC1600	Oropharynx	Mufson (26)
	CH20247	Oropharynx	Canchola (26)
M. salivarium	PG20	Oropharynx	Edward (17)
	CH643045	Oropharynx	Canchola
	CH642585	Oropharynx	Canchola
	CH1091	Oropharynx	Canchola
M. pneumoniae	FH	Oropharynx	Liu (5)
	1428	Oropharynx	Chanock (7)
	FV-3-63	Oropharynx	Baernstein (2)

Medium and cultivation techniques. Mycoplasma medium, conditions for cultivating the organisms, and method of harvesting cultures have been described (19). In general, each *Mycoplasma* strain was grown in 6-liter quantities of media prepared by Hayflick's formula (10) modified for fermentative strains to contain 1% glucose and 0.002% phenol red. Each mycoplasma preparation was examined for bacterial contamination.

DNA preparation and buoyant density determination. DNA was obtained from each mycoplasma by a minor modification of the method described by Marmur (13).

Buoyant densities in CsCl (14) were determined by Kurt Kohn, National Cancer Institute. Samples of approximately 1.5 μg of DNA were run in an analytical ultracentrifuge (Spinco, model E) at 44,770 rev/min for 20 hr at 25 C. The CsCl solutions contained 0.01 M tris(hydroxymethyl)aminomethane–0.01 M ethylenediamine-tetraacetate (*p*H 8). Band positions were measured from densitometric tracings (Joyce-Loebl microdensitometer) of ultraviolet photographs on Kodak commercial film. The buoyant density, ρ, with respect to known reference DNA samples was calculated as described by Schildkraut et al. (23). Reference values of buoyant densities of DNA from *Micrococcus lysodeikticus*, *Bacillus subtilis*, and *Clostridium perfringens* were taken as 1.7312, 1.7029, and 1.6909 g/cc, respectively. The probable error of the buoyant density differences was estimated to be $\pm$0.0005 g/cc, and the last digit of each determination is shown for comparative purposes only.

Formation and detection of DNA-RNA hybrids. Methods for synthesis of radioactive complementary RNA and a description of conditions employed for the formation and detection of DNA-RNA hybrids have been described in detail (19, 21). In brief, *Mycoplasma* DNA was employed as a template (primer) for synthesis of radioactive RNA in vitro. This complementary RNA was synthesized by the use of RNA polymerase (obtained from *Escherichia coli* B) and tritiated ribonucleoside triphosphates. The tritium-labeled RNA (and yeast RNA) was then reacted with thermally denatured template DNA or denatured DNA from other *Mycoplasma* strains and was incubated for 8 or 16 hr at 67 C. The mixture was then filtered through a nitrocellulose membrane filter (0.40 to 0.45 μ pore size). This procedure separated radioactive DNA-RNA hybrids, which are retained on the filter, from unbound RNA and RNA-RNA hybrids (18). The radioactivity retained by the filter (assayed in a liquid scintillation spectrometer) is a function of the degree of homology or genetic relatedness between nucleic acids.

Assay of genetic relatedness by the DNA-RNA homology technique. Genetic relatedness among serologically closely related mycoplasmas was determined by (i) synthesizing radioactive complementary RNA preparations with DNA from each strain as template and (ii) reacting each RNA preparation with denatured DNA from each strain. As in a previous report (19), the results of assays of hybrid formation were expressed as geometric mean counts. Two-way analysis of variance (on the logarithms of the net count rate) was performed to determine whether any interaction values differed significantly from 1.00. The relatedness value is identical to the square of the relatedness statistic proposed by Archetti and Horsfall (1).

TABLE 2. *Binding of Mycoplasma DNA preparations to radioactive RNA synthesized with representative strains of seven Mycoplasma species as template*

Source of *Mycoplasma* DNA	RNA[a]							
	Expt 1 *M. salivarium* PG20		Expt 2 *M. fermentans* G		Expt 3 *M. orale* type 2			
					(3a) DC1600		(3b) CH20247	
	Count/min retained (mean)	RNA bound relative to homologous DNA	Count/min retained (mean)	RNA bound relative to homologous DNA	Count/min retained (mean)	RNA bound relative to homolgous DNA	Count/min retained (mean)	RNA bound relative to homologous DNA
		%		%		%		%
M. salivarium PG20	2,050	*100*	0	0				
M. salivarium CH1091					201	10	314	8
M. fermentans G	63	3	928	*100*	76	4	118	3
M. orale type 2 DC1600	286	14	0	0	2,041	*100*	2,679	72
M. orale type 2 CH20247					1,965	96	3,739	*100*
M. pneumoniae Eaton FH	0	0	0	0	29	1	6	0
M. orale type 1 CH19299	284	13	33	3	186	9	280	7
M. hominis type 2 PG27	92	4	1	0	210	10	294	8
M. hominis type 1 V2785	207	10	0	0	185	9	317	8

	RNA[a]							
	Expt 4 *M. pneumoniae* Eaton FH		Expt 5 *M. orale* type 1 CH19299		Expt 6 *M. hominis* type 2 PG27		Expt 7 *M. hominis* type 1 V2 785[b]	
	Count/min retained (mean)	RNA bound relative to homologous DNA	Count/min retained (mean)	RNA bound relative to homologous DNA	Count/min retained (mean)	RNA bound relative to homologous DNA	Count/min retained (mean)	RNA bound relative to homologous DNA
		%		%		%		%
M. salivarium PG20	0	0	155	2	64	2	64	3
M. fermentans G	69	1	43	1	0	0	44	2
M. pneumoniae Eaton FH	8,836	*100*	0	0	6	0	8	0
M. orale type 1 CH19299	0	0	8,469	*100*	73	3	60	3
M. hominis type 2 PG27	24	0	212	3	3,829	*100*	76	3
M. hominis type 1 V2785	0	0					2,260	*100*
M. hominis type 1 Botteicher			142	2	10	0	1,724	76

[a] Radioactive RNA added per assay (in thousands of counts/min): experiment 1, 300; 2, 120; 3a, 85; 3b, 90; 4, 52; 5, 110; 6, 50; 7, 43. Incubation time (hours): experiments 1–3, 16; 4–7, 8. See text for hybrid assay procedure.

[b] Data from Reich et al. (19), with values calculated for 4 μg of DNA.

Serologically distinct mycoplasmas were studied in a similar fashion except that only one RNA was synthesized and this RNA was reacted either with its template DNA, or with DNA from serologically unrelated mycoplasmas. The arithmetic mean count of radioactive RNA retained by the membrane filter after reaction with heterologous DNA was expressed as a percentage of the counts retained with template DNA.

RESULTS

Genetic relatedness among representative strains from six human Mycoplasma species. Radioactive complementary RNA was prepared with template DNA from strains of *M. salivarium*, *M. fermentans*, *M. orale* types 1 and 2, *M. pneumoniae*, and *M. hominis* types 1 and 2. Each RNA was reacted with its denatured template DNA and with DNA from representative strains of the other *Mycoplasma* species. Each species was easily distinguished from the others (Table 2). In contrast, nucleic acids from two different strains of the same species (*M. orale* type 2 strains DC1600 and CH20247) gave 96 and 72% cross-reactions. Thus, the two *M. orale* type 2 isolates were much more

closely related to each other than to the other human species. The RNA preparations derived from these two *M. orale* type 2 isolates behaved almost identically when reacted with DNA from strains of other species.

Low-level cross-reactions were demonstrated among nucleic acids derived from *M. salivarium*, *M. orale* types 1 and 2, *M. hominis* types 1 and 2, and *M. fermentans*. Little or no cross-reaction was observed between *M. pneumoniae* nucleic acids and heterologous nucleic acids. The specificity of *M. pneumoniae* was demonstrated with both its DNA and complementary RNA.

M. salivarium and *M. orale* type 2 RNA preparations showed less specificity and higher cross-reactivity with the other *Mycoplasma* species than

TABLE 3. *Mean counts of radioactivity on filters after reaction between DNA from each of two strains of Mycoplasma orale type 2 and radioactive RNA synthesized with each of these DNA preparations as primer*

Source of template for radioactive RNA	Value	Source of *M. orale* type 2 DNA tested				Row mean count (RNA)
		DC1600		CH20247		
		Mean count	Interaction value[a]	Mean count	Interaction value	
DC1600	Observed[b]	2,038		1,962		2,000
	Expected[c]	1,857	1.10	2,153	0.91	
CH20247	Observed	2,679		3,738		3,164
	Expected	2,940	0.91	3,406	1.10	
Column (DNA)	Observed	2,337		2,708		2,516[d]

[a] The ratio of observed mean to expected mean. If a given DNA has the same proportional effect on both RNA preparations, and vice versa, the 99% confidence interval for the ratios is 0.95 to 1.05.

[b] Geometric mean of counts per minute retained on membrane filters per 4 μg of DNA tested for each set of triplicate experiments. Experimental conditions are described in Table 2.

[c] Geometric mean expected, calculated as the ratio of the product of the row and column means to the grand geometric mean.

[d] Grand geometric mean.

TABLE 4. *Mean counts of radioactivity retained on filters after reaction between DNA from each of three strains of Mycoplasma salivarium and radioactive RNA synthesized with each of these DNA preparations as primer*

Source of template for radioactive RNA[a]	Value	Source of *M. salivarium* DNA tested						Row mean count (RNA)
		CH1091		CH643045		CH642585		
		Mean count	Interaction value	Mean count	Interaction value	Mean count	Interaction value	
CH1091	Observed	4,023		3,049		2,616		3,178
	Expected	3,499	*1.15*	3,299	0.92	2,781	0.94	
CH643045	Observed	4,117		4,984		3,274		4,066
	Expected	4,476	0.92	4,220	*1.18*	3,557	0.92	
CH642585	Observed	1,414		1,290		1,371		1,357
	Expected	1,494	0.95	1,409	0.92	1,188	*1.15*	
Columns (DNA)	Observed	2,861		2,697		2,273		2,598[b]

[a] DNA (4 μg) was incubated for 16 hr (triplicate assays) with 100,000 to 200,000 counts/min of radioactive complementary RNA. The design and presentation of this experiment are analogous to those of Table 3. If a given DNA has the same proportional effect on all three RNA preparations, and vice versa, the 95% confidence interval for the ratios is 0.95 to 1.05, and the 99% confidence interval is 0.93 to 1.07.

[b] Grand geometric mean.

with RNA preparations from *M. fermentans*, *M. orale* type 1, *M. hominis* types 1 and 2. In fact, *M. salivarium* and *M. orale* type 2 RNA reacted with DNA from representatives of every other species except *M. pneumoniae*. The results of reciprocal DNA-RNA reactions generally agreed, although the magnitude of these reciprocal reactions varied.

Genetic heterogeneity among M. orale type 2 isolates. Statistical analysis of results of reciprocal reactions between nucleic acids derived from four *M. hominis* type 1 isolates has demonstrated genetic heterogeneity within this *Mycoplasma* species (19). An analysis of variance applied to the data obtained with the *M. orale* type 2 strains (Table 2, experiments 3a and 3b) revealed significant differences in yield between reactions involving homologous and heterologous nucleic acids. Table 3 shows these data expressed as geometric mean counts as well as the geometric mean counts that would be expected if each DNA combined proportionally with each of the two RNA preparations and vice versa. If the two strains were identical, then the ratio of the observed mean count to the expected, the interaction value,

TABLE 5. *Mean counts of radioactivity retained on filters after reaction between DNA from each of three strains and three passage levels of Mycoplasma pneumoniae and radioactive RNA synthesized with each of these DNA preparations as primer*

Expt. no.	Source of template for radioactive RNA	Value	Strain of *M. pneumoniae* tested						Row mean count (RNA)
			FH		1428		FV-3-63		
			Mean count	Interaction value[a]	Mean count	Interaction value	Mean count	Interaction value	
1[b]	FH	Observed	3,273		2,710		3,158		3,037
		Expected	3,347	0.98	2,685	1.0	3,119	1.01	
	1428	Observed	11,585		9,341		11,368		10,715
		Expected	11,806	0.98	9,471	0.99	11,003	1.03	
	FV-3-63	Observed	8,606		6,657		7,357		7,497
		Expected	8,260	1.04	6,627	1.01	7,698	0.96	
		Column mean observed	6,885		5,523		6,417		6,249[d]
	Source of template for radioactive RNA	Value	Passage level of *M. pneumoiae* FH DNA tested						Row mean count (RNA)
			Pass 15		Pass 274		Pass 299		
			Mean count	Interaction value	Mean count	Interaction value	Mean count	Interaction value	
2[c]	Pass 15	Observed	10,500		8,648		3,886		7,066
		Expected	10,554	1.00	8,714	0.99	3,837	1.01	
	Pass 274	Observed	25,668		22,035		9,945		17,785
		Expected	26,558	0.97	21,928	1.01	9,656	1.03	
	Pass 299	Observed	17,406		13,861		5,836		11,207
		Expected	16,738	1.04	13,820	1.00	6,086	0.96	
		Column mean observed	16,742		13,823		6,087		11,210[d]

[a] Analysis of variance revealed no significant differences in yield between homologous and heterologous reactions.

[b] DNA (4 μg) was incubated for 16 hr (triplicate assays) with 30 ± 5 thousand counts/min of radioactive complementary RNA.

[c] DNA (4 μg) was incubated (quadruplicate assays) for 16 hr with 165 ± 25 thousand counts/min of radioactive RNA. This experiment was performed at a later date than experiment 1.

[d] Grand geometric mean.

should fall within a confidence interval centered about 1.00. The interaction values (0.91 and 1.10) were outside the 99% confidence interval (0.95 to 1.05) for indistinguishable nucleic acid preparations, thus indicating that the two strains were different.

Genetic heterogeneity among M. salivarium isolates. M. salivarium strains were also examined for evidence of genetic heterogeneity, since earlier gel diffusion studies demonstrated small differences between two strains of this species (28). Interaction values calculated for DNA-RNA hybrids formed from three *M. salivarium* isolates were at or outside the 99% confidence interval for indistinguishable nucleic acid preparations (Table 4). Relatedness values (0.62 to 0.67) were outside the lower 99% confidence limit (0.81) for indistinguishable nucleic acid preparations. Therefore, the three strains are distinct.

Relatedness of DNA preparations from three strains of M. pneumoniae and the effect of passage history on genetic relatedness. We examined three strains of *M. pneumoniae*, a proven human pathogen, for evidence of genetic heterogeneity. These strains were obtained from different locales in different years. In addition, we tested several passage levels of the *M. pneumoniae* FH strain for genetic continuity throughout the passage history. Interaction values calculated for DNA-RNA reactions between nucleic acids derived from *M. pneumoniae* strains FH, 1428, and FV-3-63 were close to 1.00 (Table 5, experiment 1). Analysis of variance applied to these data showed that these nucleic acids were indistinguishable from each other. Similar results were obtained with the three passage levels (passages 15, 274, and 299) of the FH strains; that is, interaction values for all DNA-RNA reactions were closely grouped around 1.00 (Table 5, experiment 2). There was no evidence of genetic heterogeneity in either experiment.

Mycoplasma DNA buoyant densities. With the exception of *M. pneumoniae*, the buoyant densities of DNA from representative strains of the human *Mycoplasma* species ranged from 1.6857 to 1.6921 g/cc (Table 6). These values suggest GC/(GC + AT) ratios of 26 to 32% (23). The density of *M. pneumoniae* DNA was 1.7009 g/cc (40% GC), a value which substantially agrees with that given by other workers (16, 23). The DNA from the nonhuman strains, *M. laidlawii* and *M. gallisepticum*, had densities intermediate between *M. pneumoniae* and the other human strains.

TABLE 6. *Buoyant densities of deoxyribonucleic acids isolated from Mycoplasma strains*

Source of *Mycoplasma* DNA	Buoyant density
	g/cc
M. hominis type 1	
Genital strain PG21	1.6857
Oral strain DC63	1.6869
Oral strain Botteicher	1.6868
Oral strain V2785	1.6870
M. hominis type 2 PG27	1.6921
M. fermentans G	1.6872
M. orale type 1 CH19299	1.6869
M. orale type 2 DC1600	1.6864
M. salivarium PG20	1.6878
M. pneumoniae FH	1.7009
M. laidlawii PG9	1.6931
M. gallisepticum S6	1.6940

DISCUSSION

The nucleic acid homology technique provides an independent and quantitative means of classifying human *Mycoplasma* isolates. It offers a direct approach to microbial taxonomy, since it depends on nucleic acid base sequence complementarity rather than immunological characteristics of gene products, such as enzymes or antigenic proteins, lipids, or polysaccharides. Measurements of the immunological properties of these gene products requires production of antisera, with all the inherent complications of animal immunization. In addition, it is often difficult to identify specific antigens in mixtures which also contain cross-reactive antigens.

The results obtained with the homology method are in agreement with proposed *Mycoplasma* classifications. Seven human *Mycoplasma* species which are serologically distinct (3) can be distinguished by the homology technique. In addition, low-level cross-reactions between species, similar to those shown by complement-fixation (CF) or gel diffusion (25, 26), were revealed by the homology method. For example, serological cross-reactivity between *M. orale* type 2 isolates and five other human species (26) paralleled reactivity between *M. orale* type 2 RNA and DNA preparations from the same five species. This cross-reactivity was demonstrated with two *M. orale* type 2 isolates; the results with strain CH20247 RNA agreed within 2% with those obtained with strain DC1600 RNA. The lack of reactivity between *M. pneumoniae* DNA or RNA and nucleic acids from the other human species was also similar to serological findings (27, 28). The overall results with the homology technique were consistent with the classification proposed by Edward and Freundt (8), and extended by us and others to include more recent isolates (4, 6, 25, 26).

The ability to distinguish between *M. hominis*

TABLE 7. *Compilation of DNA buoyant densities and relatedness values for Mycoplasma species*[a]

Species	*M. pneumoniae*	*M. hominis* type 2 (*arthritidis*)	*M. salivarium*	*M. fermentans*	*M. orale* type 1	*M. orale* type 2	*M. hominis* type 1	Tissue culture isolates
M. pneumoniae (1.701)[b]	*1.01–1.12*							
M. hominis type 2 (1.692)	$<1.00 \times 10^{-4}$	NA						
M. salivarium (1.688)	$<1.00 \times 10^{-4}$	0.80×10^{-3}	*0.62–0.67*					
M. fermentans (1.687)	$<1.00 \times 10^{-4}$	$<1.00 \times 10^{-4}$	$<1.00 \times 10^{-4}$	NA				
M. orale type 1 (1.687)	$<1.00 \times 10^{-4}$	0.90×10^{-3}	0.26×10^{-2}	0.30×10^{-3}	NA			
M. orale type 2 (1.686)	$<1.00 \times 10^{-4}$*	0.81×10^{-2}*	0.13×10^{-1}	$<1.00 \times 10^{-4}$	0.64×10^{-3}*	*0.69*		
M. hominis type 1 (1.686–1.687	$<1.00 \times 10^{-4}$	$<1.00 \times 10^{-4}$	0.30×10^{-2}	$<1.00 \times 10^{-4}$	0.60×10^{-3}	0.72×10^{-2}*	*0.17–0.60*	
Tissue culture isolates[c] (1.684–1.686)	$<1.00 \times 10^{-4}$*	0.90×10^{-3}*	1.00×10^{-4}*	1.00×10^{-4}*	ND[d]	ND	1.00×10^{-4}*	*0.68–1.12*

[a] Interspecies relatedness values were calculated from the data in Table 2. Values with an asterisk were estimated by squaring the relative binding value for the one DNA-RNA reaction assayed. Italicized numbers represent the relatedness value or range of values for two or more strains within the same *Mycoplasma* species or group [see data in Results and (19) and (24)]. NA indicates that we assayed only one strain within a species.

[b] DNA buoyant density (g/cc).

[c] Data for these mycoplasmas were obtained from (19) and (24).

[d] Not determined.

type 1 isolates that were essentially identical by CF or gel diffusion, or both, was first demonstrated by the homology technique (19). This work has now been extended to include *M. salivarium* and *M. orale* type 2 isolates. More strains must be tested to determine the extent of genetic heterogeneity within these species.

The inability to differentiate among three *M. pneumoniae* isolates, obtained in different years and from several different locales, was unexpected, since heterogeneity had been demonstrated within three other *Mycoplasma* species. The serological uniqueness of this organism among human mycoplasmas has been shown previously (3, 27, 28). For these reasons, and because of the high buoyant density of its DNA, *M. pneumoniae* appears to have the fewest properties in common with the other human mycoplasmas.

High passage levels of *M. pneumoniae* are easier to cultivate in artificial media than the earlier passages. This phenomenon was not reflected in nucleic acid differences measured by the homology method. Since genetic homogeneity among strains and subsequent passages was demonstrated, it would seem that large-scale production of a vaccine against *M. pneumoniae* infections is feasible.

Except for *M. pneumoniae*, the buoyant densities of DNA preparations from human mycoplasmas fall within a relatively small range. Along with certain bovine and caprine mycoplasmas, these values are among the lowest reported. As shown in the tabular summary of relatedness values and DNA buoyant densities (Table 7), *Mycoplasma* strains with different densities were, in every case, distinguishable by the homology technique. Furthermore, relatedness values for strains within a species were greater than values for strains representative of different human *Mycoplasma* species. Mycoplasmas with identical densities were, in some cases, distinguished from each other. Thus, although density determinations yield information about overall DNA base compositions, the homology technique permits finer discrimination between *Mycoplasma* DNA preparations.

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