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Genetic Relatedness Among Mycoplasmas as Determined by Nucleic Acid Homology

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ABSTRACT

REICH, PAUL R. (National Institutes of Health, Bethesda, Md.), NORMAN L. SOMERSON, JAMES A. ROSE, AND SHERMAN M. WEISSMAN. Genetic relatedness among mycoplasmas as determined by nucleic acid homology. J. Bacteriol. **91**:153–160. 1966.—A sensitive membrane filter method to detect nucleic acid homology was used to determine genetic relatedness among mycoplasma isolates. Deoxyribonucleic acid (DNA) was isolated from mycoplasmas and used as a primer for synthesis of tritium-labeled, complementary ribonucleic acid (RNA) by the enzyme RNA polymerase. DNA from each mycoplasma isolate tested was reacted separately with complementary RNA synthesized with homologous or heterologous DNA as primer. The quantity of DNA-RNA hybrids formed was assayed by the nitrocellulose membrane filter method. The amount of radioactivity bound to the membrane filter was used to measure the degree of homology between the nucleic acids. The three mycoplasma isolates from human oral cavities (DC 63, V2785, Botteicher) and the prototype strain PG21 placed in the *Mycoplasma hominis* type 1 group by gel diffusion and complement-fixation testing were investigated with this technique. Analysis of the data confirmed their immunological grouping with the *M. hominis* type 1 and their distinction from other human mycoplasmas. In contrast to the data from immunological studies, none of the four isolates tested appeared to be identical to any other. Preliminary experiments with DNA from four other mycoplasma isolates from tissue cultures inoculated with human material revealed them to be closely related, and possibly identical. The advantages of this nucleic acid homology technique for the study of relatedness among mycoplasmas are described.

Deoxyribonucleic acid (DNA) homology techniques provide a new approach to the assessment of relatedness among microorganisms (15). These methods have been used to study relationships among bacterial and mammalian species (11, 15). A sensitive membrane filter method to detect homology (20) and a method of synthesizing in vitro complementary, radioactive ribonucleic acid (RNA; 4) offer the opportunity to investigate genetic relatedness among mycoplasma strains where only microgram quantities of DNA are available.

A classification of a number of human mycoplasma strains has been suggested by Edward and Freundt (6) and supported by immunological data (13, 23). The *Mycoplasma hominis* type 1 group was immunologically distinct from the other classified human mycoplasmas. Gel diffusion and complement-fixation testing by Taylor-Robinson et al. (23), placed three mycoplasma isolates in the *M. hominis* type 1 group. The three oral strains V2785, DC63, and Botteicher (Botte) were indistinguishable by these serological techniques and were closely related to the prototype PG21, a genital strain. However, these four strains differed from one another in growth characteristics and morphology (Somerson and Chanock, *unpublished data*).

The classification of many strains of serologically related mycoplasmas recently isolated from tissue cultures inoculated with tumor material is uncertain (2, 3, 8, 9). A comparison of these new isolates and the *M. hominis* type 1 strains by use of nucleic acid hybridization techniques was performed to complement the serological classification and to provide information

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TABLE 1. *Source of Mycoplasma strains*

Serological type or species	Strain	Recovered from	Source of culture (reference)
Human strains			
M. hominis type 1	PG-21	Genital tract	Edward (19)
	Botte	Oropharynx	Mufson (23)
	V2785	Oropharynx	Mufson (23)
	DC63	Oropharynx	Mufson (18)
M. hominis type 2	Campo, PG-27	Genital tract	Edward, originally from Dienes
M. salivarium	PG-20	Oropharynx	Edward (19)
*M. fermentans**	G	Genital tract	Edward (21)
*M. pneumoniae**	FH	Oropharynx	Liu (5)
M. orale	CH19299	Oropharynx	Canchola (22)
Turkey strain			
*M. gallisepticum**	S 6	Brain	Madden (24)
Sewage strain			
*M. laidlawii**	PG-9	Sewage	Edward (12)
Unclassified tissue culture isolates*	F-7†	Tissue culture fluids‡	Somerson and Lewis, *unpublished data*
	F-11 & F-12	Tissue culture fluids	Hayflick (2)
	F-13	Tissue culture fluids	Somerson and Smith, *unpublished data*
	Negroni agent	Tissue culture fluids	Somerson and Lewis (8)
	880	Tissue culture fluids	Horoschewitz (9)

* Glucose-fermenting strains.

† Cincy tumor 7.

‡ Mycoplasmas isolated from serial "fluid" passages of tissue cultures inoculated with human material.

on the validity and potential of this physical-chemical approach. The results, although substantially in agreement with the available immunological data, reveal certain differences among the *M. hominis* type 1 isolates.

MATERIALS AND METHODS

Organisms. The prototype human mycoplasma strains, isolates from the oropharynx, and unclassified tissue culture isolates are listed in Table 1. Their serological relationships have been discussed elsewhere (22, 23).

Media and cultivation techniques. The mycoplasma medium was the same as used for the isolation of *M. pneumoniae* (5, 10), but was modified to contain 1% glucose and, for fermentative strains, 0.002% phenol red. Mycoplasma broth media were inoculated with samples of cultures that had been sealed in ampoules and stored at −70 C. The mycoplasmas were then subcultured at 3- to 6-day intervals through several serial passages before inoculation into large quantities of medium. The fermentative strains were grown in 500 ml of mycoplasma broth contained in 2-liter Povitsky bottles (Diphtheria Toxin Bottles, Phipps and Bird, Richmond, Va.), since they appeared to grow better in containers which have a large surface area and permit a shallow culture medium layer. Mycoplasmas which did not ferment sugar were grown in 500-ml quantities of mycoplasma medium contained in Kimler screw-capped bottles. Each mycoplasma strain was grown in 6-liter quantities and incubated at 36 C for 3 to 7 days. The duration of incubation was based upon earlier experiments which determined the incubation period necessary for maximal viability. The mycoplasmas were concentrated by centrifugation (30,000 × *g*) in a refrigerated (4 C) Servall continuous-flow system.

Mycoplasma DNA preparation. The concentrated mycoplasmas were lysed by addition of 2% sodium dodecyl sulfate and incubation at 37 C for 10 to 15 min. DNA was obtained by a minor modification of the method described by Marmur (16). After treatment with pancreatic ribonuclease (20 μg/ml; Sigma Chemical Co., St. Louis, Mo.), 20 μg/ml of subtilisin (Enzyme Development Corp., New York, N.Y.) was used for an additional 30 min at 37 C to remove traces of ribonuclease.

The DNA content of solutions was estimated from the absorption of ultraviolet light at 260 mμ. An optical density of 0.025 was assumed to be equivalent to a DNA concentration of approximately 1 μg/ml.

Radioactive substrates. The following tritiated nucleoside triphosphates were obtained from Schwarz BioResearch Inc., Orangeburg, N.Y.: adenosine triphosphate-H^3 (ATP), cytosine triphosphate-H^3,

guanosine triphosphate-H^3, and uridine triphosphate-H^3 (specific activity, 2.5, 1.1 or 2.5, 1.1, and 3.2 c/mmole, respectively). The final concentration of these triphosphates in aqueous solution was 2.0 mmoles/ml after evaporation at 4 C.

Radioactive RNA production. Frozen *Escherichia coli* B cells (General Biochemicals, Chagrin Falls, Ohio) were used to prepare RNA polymerase (4). The activity of the final diethylaminoethyl (DEAE) cellulose eluate was such that 0.01 ml incorporated 5.5 mμmoles of ATP per 10 min with standard incubation conditions. The enzyme was stored in liquid nitrogen (−196 C).

Radioactive RNA, which forms specific complexes with complementary DNA (DNA-RNA hybrids or duplexes; 7) was synthesized under conditions described by Chamberlin and Berg (4). Approximately 20 μg of primer DNA, 20 mμmoles of each radioactive nucleoside triphosphate, and 0.04 to 0.05 ml of enzyme solution were used. After incubation for 20 min, 1.5 μg of ribonuclease-free deoxyribonuclease (Worthington Biochemical Corp., Freehold, N.J.) was added, and the incubation was continued for 15 min. The radioactive RNA was isolated by a hot phenol, sodium dodecyl sulfate procedure described elsewhere (Rose, Reich, and Weissman, Virology, *in press*). The final precipitate was dissolved in 0.015 M NaCl-0.0015 M sodium citrate (*p*H 6.7). To measure acid-precipitable radioactivity, a 0.005-ml portion was added to 3 ml of 5% trichloroacetic acid and poured over a B-6 membrane filter (Schleicher & Schuell Co., Keene, N.H.). The filter was washed with 10 ml of 5% trichloroacetic acid and assayed for radioactivity as described below.

Formation and detection of DNA-RNA hybrids. These methods are described elsewhere in detail (Rose, Reich, and Weissman, *in press*). Briefly, a similar quantity of each DNA to be tested was incubated with an identical amount of radioactive RNA and approximately 40 μg of yeast RNA as carrier. The incubation mixture also contained 0.3 M NaCl, 0.03 M sodium citrate (*p*H 6.7), and 50 μg of subtilisin in a 0.10-ml total volume. Subtilisin was used to destroy residual ribonuclease or deoxyribonuclease activity. The mixtures were incubated at 100 C for 10 min to denature the DNA. The temperature was held at 67 C for 8 hr to allow DNA-RNA duplex formation. These conditions were found to be optimal for the formation of *E. coli* DNA-RNA complexes, and are similar to those used by others (15, 20).

The sample was diluted with 0.5 M NaCl–0.01 M tris(hydroxymethyl)aminomethane (*p*H 7.3), and was filtered through a membrane filter. Radioactivity retained on the filter was proportional to the amount of RNA complexed with complementary DNA (20). To determine the amount of nonspecifically retained radioactivity, reaction mixtures were incubated and processed without DNA. The radioactivity nonspecifically bound represented less than 0.06% of the radioactivity added to the vial. Corrections were made for variations in the amount of radioactive DNA and RNA added to each vial. All assays were performed in duplicate.

Assay of radioactivity. Filters were washed with 25 ml of 80% ethyl alcohol and dried. The filters were assayed in liquid scintillation counters (Packard Instrument Co., La Grange, Ill.) with plastic counting

TABLE 2. *Binding of Mycoplasma DNA to radioactive RNA synthesized with M. hominis type 1 strain V2785 as primer*

Source of *Mycoplasma* DNA	Quantity of DNA tested	Counts/min in RNA retained (mean)	Counts per min retained per μg of DNA	RNA bound relative to V2785 DNA*
	μg			%
M. hominis type 1				
Oral strains				
V2785	11	6,209	565	100
Botte	10	4,311	431	76
DC63	11	2,442	222	39
Genital strain				
PG 21	17	6,757	398	70
M. hominis type 2 PG 27	11	216	19	3
M. salivarium PG 20	10	156	16	3
M. orale CH19299	19	281	15	3
M. fermentans G	8	87	11	2
M. pneumoniae Eaton-FH	12	24	2	0
M. laidlawii PG 9	10	12	1	0
Escherichia coli (control) B	10	25	3	0

* DNA and 43,000 counts ± 4,000 counts/min of radioactive RNA synthesized with RNA polymerase and strain V2785 DNA as primer were added to duplicate vials. The vials in this and succeeding experiments were processed under conditions (see text) necessary for formation and detection of DNA-RNA hybrids. The arithmetic mean counts per minute of radioactive RNA retained by the membrane filter per microgram of strain V2785 DNA was set equal to 100%. The results of similar calculations for each DNA were expressed as percentage of the counts per minute per microgram of DNA retained with strain V2785 DNA.

TABLE 3. *Mean counts of radioactivity retained on filters after reaction between DNA from each of four strains of Mycoplasma hominis type 1 and radioactive RNA synthesized with each of these DNA preparations as primer*

Source of primer for radioactive RNA[a]	Value	Source of *M. hominis* type 1 DNA tested								Row mean count (RNA)
		PG 21		DC 63		Botte		V2785		
		Mean count	Interaction value[b]	Mean count	Interaction value	Mean count	Interaction value	Mean count	Interaction value	
PG 21	Observed[c]	2,243		1,120		1,374		1,607		1,535
	Expected[d]	1,786		1,262		1,474		1,673		
			1.26		0.89		0.93		0.96	
DC 63	Observed	1,630		1,535		1,269		1,289		1,422
	Expected	1,654		1,169		1,365		1,550		
			0.99		*1.31*		0.93		0.83	
Botte	Observed	1,386		1,087		1,634		1,353		1,351
	Expected	1,572		1,111		1,297		1,472		
			0.88		0.98		*1.26*		0.92	
V2785	Observed	1,497		1,013		1,237		2,087		1,407
	Expected	1,637		1,157		1,351		1,533		
			0.91		0.88		0.92		*1.36*	
Column (DNA) mean count	Observed	1,660		1,173		1,370		1,555		

[a] DNA (4 μg) was incubated with 125,000 counts/min ± 10,000 counts/min of tritium-labeled RNA synthesized with RNA polymerase and mycoplasma DNA as primer.

[b] The ratio of observed mean to expected mean. If a given DNA has the same proportional effect on all four RNA preparations, and vice versa, the 95% confidence limits for the ratios are 0.95 to 1.05 and 99% confidence limits are 0.93 to 1.05.

[c] Geometric mean of counts per minute retained on membrane filters per microgram of DNA tested for each set of duplicate experiments.

[d] Geometric mean expected, calculated as the ratio of the product of the row and column means to the grand geometric mean (1,427), e.g., PG21 DNA and DC 63 RNA, $1,654 = \frac{1,422 \times 1,660}{1,427}$.

vials and Liquifluor scintillation solution (Nuclear-Chicago Corp., Des Plaines, Ill.). The efficiency of counting was approximately 15% with a background of 19 to 21 counts/min. The statistical counting error was less than ±4%.

Statistical analysis. Logarithms were taken of the individual counts, and the resulting two-way layout of data (two observations per cell) was subjected to the usual analysis of variance. Confidence intervals were calculated for contrasts among interactions only if the interaction mean square significantly exceeded the residual mean square. Results were expressed in the original scale as geometric means and as confidence intervals based on the log normal distribution. It is readily shown that the relatedness statistic used by us is identical to the square of the relatedness statistic proposed by Archetti and Horsfall (1).

RESULTS

Relationship of M. hominis type 1 strain V2785 DNA to DNA from other human mycoplasmas. RNA was synthesized with strain V2785 DNA as primer and was then reacted with DNA from other mycoplasma strains under conditions optimal for DNA-RNA hybrid formation and detection. The quantity of radioactivity retained on the membrane filters was expressed as a percentage of the counts per minute per microgram retained with strain V2785 DNA (Table 2). There was a 39% or greater reaction with the three other *M. hominis* type 1 strains and 3% or less reaction with DNA from *M. fermentans*, *M. pneumoniae*, *M. orale*, and from the saprophyte *M. laidlawii*. No radioactivity was retained with the *E. coli* B control. Thus, the four strains of the *M. hominis* type 1 group appeared to be more closely related to each other than to the other human mycoplasma species.

Relatedness among M. hominis type 1 isolates. To estimate the magnitude of relatedness among the four *M. hominis* type 1 strains, RNA was synthesized with primer DNA from each of these isolates and tested with DNA from each strain for

TABLE 4. *Mean counts of radioactivity retained on filters after reaction between DNA from one of two preparations of strain Botte or PG21 and radioactive RNA synthesized with one of these DNA preparations as primer*

Source of primer for radioactive RNA*	Value	Source of *M. hominis* type 1 DNA tested						Row mean count (RNA)
		Botte prepn 1		Botte prepn 2		PG-21		
		Mean count	Interaction value	Mean count	Interaction value	Mean count	Interaction value	
Botte prepn 1	Observed	2,445		1,371		1,473		1,703
	Expected	2,400		1,211		1,699		
			1.02		1.13		0.87	
Botte prepn 2	Observed	2,802		1,344		1,536		1,795
	Expected	2,530		1,276		1,790		
			1.11		1.05		0.86	
PG 21	Observed	1,483		709		1,593		1,188
	Expected	1,674		845		1,185		
			0.89		0.84		1.34	
Column (DNA) mean count	Observed	2,616		1,093		1,533		

* DNA (4 μg) was incubated with 73,000 counts/min $\pm$ 7,000 counts/min of tritium-labeled RNA synthesized with RNA polymerase and mycoplasma DNA as primer. The design and presentation of this experiment is analogous to that of Table 3. If a given DNA has the same proportional effect on all four RNA preparations, and vice versa, the 95% confidence interval for the ratios is 0.85 to 1.18 and the 99% confidence interval is 0.80 to 1.24 (grand geometric mean = 1,537).

its ability to form DNA-RNA hybrids. The results are entered in Table 3 as observed geometric mean counts, each mean representing a duplicate experiment. Also shown are the geometric mean counts that would be expected if each DNA had the same proportional effect on all four RNA preparations and vice versa, i.e., if there were no effects other than those expressed by the row and column geometric mean counts [row (RNA) effects and column (DNA) effects, respectively]. If only row and column effects are present, then the ratio of the observed mean count to the expected, the interaction value, should fall within a confidence interval centered about 1.00. All of the interaction values for homologous DNA-RNA hybrids (e.g., PG21 DNA and RNA primed by PG21 DNA) were greater than 1.13, the upper 99% confidence limit. On the other hand, all of the interaction values of heterologous DNA-RNA hybrids (e.g., PG21 DNA and RNA primed by Botte DNA) fell below 1.00. This pattern indicates an appreciable difference in yield between reactions involving homologous and heterologous nucleic acids derived from *M. hominis* type 1 strains. In no case was there a statistically significant difference between reciprocal tests (e.g., PG21 DNA and RNA primed by Botte DNA as compared with Botte DNA and RNA primed by PG21 DNA).

The degree of similarity between two DNA preparations from *M. hominis* type 1 group can be expressed as a relatedness value, or ratio of the product of the heterologous interaction values to the product of the homologous interaction values (1). For example, the PG21 DNA and Botte DNA relatedness value equals the product of the PG21 DNA-Botte RNA and the Botte DNA-PG21 RNA interaction values divided by the product of the PG21 DNA-PG21 RNA and Botte DNA-Botte RNA interaction values. By definition, a relatedness value of 1.00 implies that the DNA preparations are identical. In this experiment, the relatedness values ranged between 0.41 and 0.55, and none was within the 99% confidence limits calculated for identical DNA preparations (0.83 to 1.21). Therefore, the four strains in the *M. hominis* type 1 group were not identical.

Assay of DNA of identical mycoplasma origin. A control experiment was designed to demonstrate that DNA from two preparations of the same mycoplasma are indistinguishable by the DNA-RNA homology technique. DNA prepared on two occasions from different cultures of *M. hominis* type 1 strain Botte and DNA from prototype strain PG21 were used as primers of radioactive RNA synthesis. The synthesized RNA preparations were incubated separately with the Botte and PG21 DNA under conditions necessary

TABLE 5. *Binding of mycoplasma DNA to radioactive RNA synthesized with unclassified strain F-7 DNA as primer*

Expt	Source of mycoplasma DNA	Quantity of DNA tested	Counts/min in RNA retained (mean)	Counts per min retained per μg of DNA	RNA bound relative to strain F-7
		μg			%
1*	Unclassified tissue culture isolates				
	F-7	20	10,186	509	100
	F-13	19	10,495	552	109
	F-12	22	11,083	504	99
	F-11	24	11,320	472	93
	Negroni agent	19	267	14	3
	880	22	12	1	0
	M. laidlawii PG 9	20	38	2	0
	M. gallisepticum S6	26	19	1	0
Escherichia coli B	Control	20	10	1	0
2†	Unclassified tissue culture isolate				
	F-7	20	7,179	359	100
	M. hominis type 2 PG 27	18	176	10	3
	M. hominis type 1, oral strain V2785	22	96	4	1
	M. salivarium PG 20	22	98	5	1
	M. fermentans G	17	76	5	1
	M. pneumoniae Eaton-FH	24	17	1	0
Escherichia coli B	Control	20	344	17	5

* DNA and 49,000 counts/min ± 5,000 counts/min of radioactive RNA synthesized with RNA polymerase and a strain F-7 (Cincinnati, tumor 7) DNA as primer were added to duplicate vials. The arithmetic mean counts per minute of radioactive RNA retained by the membrane filter per microgram of strain F-7 DNA was set equal to 100%. The results of similar calculations for each DNA were expressed as percentage of the counts per minute per microgram of DNA retained with strain F-7 DNA.

† The design of this experiment (performed at a later date) is similar to that described above, except that 57,000 counts/min ± 6,000 counts/min of radioactive RNA were used, and the incubation time was extended to 18 hr.

for formation and detection of DNA-RNA duplexes.

The interaction values (Table 4) calculated for reciprocal cross reactions between the two Botte preparations were similar, and fell within the stated 95% confidence limits for the null hypothesis described in the preceding experiment. The homologous PG21 DNA-RNA interaction value was greater than 1.24, the upper 99% confidence limit, and was much higher than values obtained for heterologous reactions with PG21 DNA or RNA ($\leq$ 0.89). The relatedness values between DNA preparations were: Botte preparation 1 and Botte preparation 2, 1.17; PG21 and Botte preparation 1, 0.56; PG21 and Botte preparation 2, 0.51. The 95% confidence limits for identical DNA preparations were 0.62 and 1.62, and 99% confidence limits were 0.52 to 1.93. These calculations confirmed the difference between strains PG21 and Botte, and demonstrated that the two Botte preparations cannot be distinguished from each other.

Relationship of DNA from mycoplasma strain F-7 to DNA from other unclassified isolates and from other mycoplasma groups. DNA was obtained from a number of unclassified, serologically related mycoplasmas (Table 1). RNA was synthesized with primer DNA from strain F-7 and reacted with DNA from mycoplasma strains F-7, F-11, F-12, F-13, Negroni agent, 880, *M. laidlawii*, and *M. gallisepticum*. The quantity of radioactivity retained on the membrane filters was expressed as a percentage of the counts per minute per microgram retained with DNA from isolate F-7 (Table 5). There were distinct reactions (93 to 109%) with DNA from F-11, F-12, F-13, and there was 3% or less reaction with Negroni agent, strain 880, *M. laidlawii*, *M. gallisepticum*, and *E. coli* B. Preliminary serological evidence confirms the relatedness among F-7, F-11, F-12,

and F-13 isolates and their distinction from Negroni agent and strain 880 (2).

DNA from mycoplasmas of human origin were similarly tested with another preparation of this RNA (Table 5). Reactions were 3% or less with all human strains tested. The 5% value obtained with *E. coli* B represents either short segments of homology with mycoplasma DNA, or, more likely, contamination of this sample of RNA polymerase with DNA from *E. coli* B. The prolonged incubation time used in this experiment would not affect the relative amounts of radioactive RNA retained on the membrane filters (Reich, *unpublished data*).

DISCUSSION

The demonstration of DNA-RNA hybrid formation between DNA isolated from strains DC63, Botte, and PG21 and RNA synthesized with strain V2785 DNA as primer was expected from examination of available immunological data. Complement-fixation testing, gel diffusion, and immunofluorescence techniques indicate that strains DC63, Botte, and V2785 were indistinguishable and were related to the prototype PG21. Strain V2785 was easily distinguished from the other human mycoplasma species by both immunological and physical-chemical techniques.

However, when RNA synthesized with primer DNA from each of the four *M. hominis* type 1 strains was reacted separately with each DNA, the reactions between homologous nucleic acids produced yields significantly in excess of those which would be expected on the basis of proportional effects between DNA and RNA. These excesses (interactions) may result from the specific and close complementation between their base sequences (7, 17). The techniques of statistical analysis accounted for errors in estimating DNA concentration by ultraviolet light absorption, variation in the ability of a given DNA or RNA preparation to form DNA-RNA hybrids, and other "row and column effects." Thus, these factors cannot explain these highly significant interactions.

The determination of relatedness values permitted calculation of the probability that two DNA preparations from mycoplasmas were not identical. With *M. hominis* type 1 isolates, all relatedness values fell outside the 99% confidence limits calculated for identical DNA preparations. A control experiment demonstrated that DNA from two different preparations of the same mycoplasma appeared identical when assessed by this technique.

These results suggest that differences in growth characteristics and morphology among these strains are a reflection of substantial differences in their genetic material. Many immunological methods previously utilized appear to be less specific than the DNA-RNA homology technique. The latter not only allows detection of gross differences among mycoplasma species, but also can detect fine differences within groups of related organisms.

An investigation of DNA from the unclassified mycoplasma isolates was undertaken to define their interrelationships and cross-reactions with DNA from other mycoplasma species. On the basis of data obtained with the use of strain F-7 as primer DNA, the F-7, F-11, F-12, and F-13 strains appear to contain closely related DNA. They are readily distinguished from the *M. hominis* type 1 group and other mycoplasmas, including human, avian, and saprophytic strains. This finding presumably indicates a distant relationship between these unclassified tissue culture isolates and other mycoplasma groups. A detailed investigation analogous to that carried out with four *M. hominis* type 1 strains is required to determine whether the unclassified isolates are identical or merely related.

There are certain reservations regarding the significance of the degree of cross-reaction among related organisms. The use of RNA polymerase to synthesize RNA in vitro with a base sequence complementary to a DNA primer has been described (7). At least part of both DNA strands appear to be copied, but it is not known whether replicate copies of some segments of DNA are made. The interaction and relatedness values calculated for DNA-RNA reactions might be altered, if multiple copies of one segment of a primer DNA with base sequences uniquely shared with DNA from another organism are transcribed into the complementary RNA. However, the relatively large fraction of radioactive complementary RNA bound by its homologous DNA suggests that large portions of the base sequences in the primer DNA have been transcribed into complementary RNA.

The advantages of the DNA-RNA homology technique used in these experiments include the requirement for only microgram quantities of DNA, greater sensitivity to small differences in mycoplasmas compared with some immunological methods, and its suitability for application of standard statistical techniques. The problems of animal immunization for production of antibodies and the possibility of nonspecific serological cross-reactions to mycoplasma broth medium constituents are completely avoided. Detection of mycoplasma infections of mammalian cells in tissue culture or in tumor material is a possibility,

since the question of mycoplasma contamination during tissue culture passage of such materials does not arise.

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