Deoxyribonucleic Acid Base Compositions of Mycoplasma Strains of Avian Origin

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(Accepted for publication 11 December 1968)

SUMMARY

The guanine + cytosine (GC) base compositions of deoxyribonucleic acids (DNA) of avian mycoplasmas, as well as strains from other sources, were determined from buoyant density in CsCl. The values from our work ranged from 24.0 to 35.7 moles %. There were small clusters at about 35 % and 32 % but the rest of the values ranged continuously from 24.0 % to 30.5 %. From reports in the literature, the values for the Mycoplasma group ranged from 22.8 to 41.0.

INTRODUCTION

Knowledge of the mean base composition of deoxyribonucleic acid (DNA) of organisms is an important criterion in classifying species. These techniques, however, have not been applied to the avian mycoplasmas. Nucleic acid homology techniques have been used to determine the lack of relatedness between mycoplasmas and particular bacteria (McGee, Rogul, Falkow & Wittler, 1965; Rogul, McGee, Wittler & Falkow, 1965) and to show relationships among the mycoplasmas of human origin (Reich, Somerson, Hybner, Chanock & Weissman, 1966; Reich, Somerson, Rose & Weissman, 1966). DNA studies were reported at the Second Conference on Biology of the Mycoplasma (N.Y. Academy of Sciences) comparing mycoplasma strains with each other and with L-forms and bacteria (McGee, Rogul & Wittler, 1967; Neimark, 1967; Somerson, Reich, Chanock & Weissman, 1967).

This study was undertaken to determine the guanine + cytosine (GC) contents of the DNA of mycoplasmas and to contribute data that may lead to more precise classification of these organisms.

METHODS

The sources of some strains have been reported (Kelton, Gentry & Ludwig, 1960; Kelton & Van Roekel, 1963). The three bovine strains were obtained from Drs Edward and Leach, the Wellcome Research Laboratories, Beckenham, Kent, England; the porcine strain, *Mycoplasma granularum*, from Dr W. Switzer, Iowa State University; *M. gallisepticum* strains 801, 1010 and 1150 from Dr H. Yoder, Iowa State

University; *M. meleagridis* strain 529 from Dr R. Yamamoto, University of California, Davis. The strains representing avian serotypes I, J, K, L, and M were obtained from Dr M. Frey, Iowa State University, and *M. synoviae* strain 1853 from Dr N. Olsen, West Virginia University. Strain 4330 is designated *M. hominis*, type 1 (Morton &

Table 1. *DNA base compositions of Mycoplasma strains of avian origin*

Species	Strain	Serotype	CsCl buoyant density (g./cm. ³)	GC content (mole %)
<i>M. gallisepticum</i>	801	A	1·695	35·7
<i>M. gallisepticum</i>	PG-31	A	1·695	35·7
<i>M. gallisepticum</i>	1010	A	1·695	35·7
<i>M. gallisepticum</i>	S-6	A	1·6945	35·2
<i>M. gallisepticum</i>	1150	A	1·694	34·7
<i>M. synoviae</i>	1853	S	1·6935	34·2
<i>Mycoplasma</i> sp.	694	L	1·6915	32·1
<i>Mycoplasma</i> sp.	TU	C	1·6899	30·5
<i>Mycoplasma</i> sp.	C	C	1·6885*	29·1
<i>M. iners</i>	O	G	1·689	29·6
<i>M. iners</i>	PG-30	G	1·6885	29·1
<i>Mycoplasma</i> sp.	PSU-4	D	1·688*	28·6
<i>Mycoplasma</i> sp.	NY	D	1·6875	28·1
<i>M. meleagridis</i>	529	H	1·688	28·6
<i>M. meleagridis</i>	N	H	1·6875	28·1
<i>M. gallinarum</i>	54-537	B	1·6865	27·0
<i>M. gallinarum</i>	PG-16	B	1·686	26·5
<i>Mycoplasma</i> sp.	SA	F	1·686	26·5
<i>Mycoplasma</i> sp.	693	J	1·685	25·5
<i>Mycoplasma</i> sp.	R-49	M	1·6845	25·0
<i>Mycoplasma</i> sp.	695	I	1·684	24·5
<i>Mycoplasma</i> sp.	1805	K	1·684	24·5
<i>Mycoplasma</i> sp.	HPR-15	E	1·6835	24·0

* Average of four determinations; all others, average of two determinations.

Table 2. *DNA base compositions of Mycoplasma strains other than avian*

Species	Strain	Origin	CsCl buoyant density (g./cm. ³)	GC content (mole %)
<i>Mycoplasma</i> sp. (saprophyte)	PG-10	Bovine	1·6935	34·2
<i>Mycoplasma</i> sp. (saprophyte)	C-15	Compost	1·692	32·7
<i>M. arthritis</i> (<i>M. hominis</i> , type 2)	07	Human	1·6915	32·1
<i>M. arthritis</i> (<i>M. hominis</i> , type 2)	39	Human	1·6915	32·1
<i>M. granularum</i>		Porcine	1·6915	32·1
<i>M. maculosum</i>	PG-15	Canine	1·689	29·6
<i>M. spumans</i>	PG-13	Canine	1·6885	29·1
<i>M. canis</i>	PG-14	Canine	1·6885*	29·1
<i>M. bovisgenitalium</i>	PG-11	Bovine	1·6875	28·1
<i>M. hominis</i> , type 1	4330	Human	1·686	27·3
<i>M. bovirhinis</i>	PG-43	Bovine	1·684	24·5

* Average of four determinations; all others, average of two determinations.

Roberts, 1967) and strains 07 and 39 are designated *M. arthritidis* (*M. hominis*, type 2) (Edward & Freundt, 1965). All strains were purified as described previously (Kelton & Van Roekel, 1963).

In general, 2 l. batches of broth medium (Kelton & Van Roekel, 1963) or more were inoculated with 10 ml. of 48 hr culture/l. and incubated at 37° on a rotary shaker at approximately 90 rev./min. until visible turbidity developed. The organisms were separated from the medium by centrifugation at 7500 g, washed once with 0.15 M-NaCl plus 0.1 M-sodium ethylenediaminetetra-acetate, and resuspended in a small volume of the same buffer. With *Mycoplasma synoviae* the broth medium was supplemented with L-cysteine hydrochloride hydrate (Calbiochem) and nicotinamide adenine dinucleotide (Pabst Laboratories) each in final concentration of 0.01 %.

The organisms were lysed by adding sodium dodecyl sulphate to 1 % (w/v) and the DNA was isolated by the method of Marmur (1961) as modified by Mandel, Bergendahl & Pfennig (1965). The base composition was estimated from buoyant density in CsCl in an analytical centrifuge (Schildkraut, Marmur & Doty, 1961). Buoyant densities were calculated relative to DNA of *Escherichia coli* at a density of 1.710 g./cm.³.

RESULTS

Buoyant densities and base compositions of DNA samples derived from strains of avian origin are shown in Table 1. Strains from other sources were included in this study: results from these strains are shown in Table 2.

The GC content of the DNA samples ranged from 24.0 to 35.7 moles %. There was a small cluster of values at about 35 % and another at 32 %, but the rest of the values formed a continuous gradation from 24.0 to 30.5 %.

DISCUSSION

In grouping micro-organisms the GC content appears to be most useful when the values are different. Similar values were obtained from mycoplasmas that are apparently unrelated. *Mycoplasmas arthritidis* (*M. hominis*, type 2) of human origin had the same GC content as *M. granularum* of porcine origin, and the value for the saprophyte, C-15, was only slightly higher. Likewise, within the avian group, serotypes A and S gave similar values and there were only minor differences among the values for serotypes B, C, D, F, G, and H. In the studies by McGee *et al.* (1967) the relationships of five strains having GC content of 32 % were studied by DNA-DNA hybridization. They found that these five strains fell into three unrelated groups and attributed the similar base ratios to chance.

Strains representing 15 serotypes of avian *Mycoplasma* were included in this study. Despite repeated attempts, DNA suitable for analysis could not be obtained from strain L 3-10, representing serotype Q.

In previous studies (Kelton & Van Roekel, 1963) serological relatedness was demonstrated between serotype E (strain HPR-15) and serotype G (strains PG-30 and O) and these strains were included in the same serotype. Dierks, Newman & Pomeroy (1967) presented similar results in their serological studies of the avian mycoplasmas. The GC contents of the DNA of these strains, however, do not indicate any relationship between these serotypes.

Table 3. *DNA base compositions of Mycoplasma strains reported in the literature*

Species	Strain and source	GC (mole %)	Method*	Reference
<i>M. pneumoniae</i>	Human	39-41	B, C	Neimark & Pene, 1965
	FH, human	39.0, 40.8	B, C	Neimark, 1967
	ATCC-15377, human	39.3	B	McGee <i>et al.</i> 1965
	BRU, ATCC-15377, human	38.6	B	McGee <i>et al.</i> 1967
<i>M. gallisepticum</i>	A 5969, avian	35.0	B	Marmur & Doty, 1962
	A 5969, avian	34.0	C	Schildkraut <i>et al.</i> 1961
	Avian	33.4, 33.7	B, C	Morowitz <i>et al.</i> 1967
	A 5969, avian	33.0-33.5	A, C	Morowitz <i>et al.</i> 1962
	ATCC-15302, avian	32.5	B	Rogul <i>et al.</i> 1965
	S-6, ATCC-15302, avian	32.0	B	McGee <i>et al.</i> 1967
<i>M. agalactiae</i>	Goat milk	33.6, 34.2	B, C	Neimark, 1967
<i>M. hominis</i>	H 39, human	33.7, 31.6	B, C	Morowitz <i>et al.</i> 1967
<i>M. hominis</i> , type 2	CAMPO, ATCC-14152, human	31.7	B	McGee <i>et al.</i> 1967
<i>M. arthritis</i>	H 606, ATCC-13988	31.9	B	McGee <i>et al.</i> 1967
<i>Mycoplasma</i> sp.	DONNETTA, bovine	30.0, 32.6	B, C	Morowitz <i>et al.</i> 1967
<i>M. laidlawii</i> , type A	ATCC-14089	32.9	B	Morowitz <i>et al.</i> 1967
		32.5	B	McGee <i>et al.</i> 1967
		31.7, 35.7	B, C	Neimark, 1967
		34.1	B	Morowitz <i>et al.</i> 1967
<i>M. laidlawii</i> , type B	SEWAGE	31.0-35.5	B, C	Neimark & Pene, 1965
	ATCC-14192	32.3	B	McGee <i>et al.</i> 1967
		33.2, 33.7	B, C	Morowitz <i>et al.</i> 1967
		30.4	B	McGee <i>et al.</i> 1967
<i>M. granularum</i>	BTS-39, ATCC-19168, porcine	29.6	B	McGee <i>et al.</i> 1967
<i>M. bovis genitalium</i>	PG-11, ATCC-14173, bovine	29.2	B	McGee <i>et al.</i> 1967
<i>M. hominis</i> , type 1	H-34, ATCC-15056, human	28.7	B	McGee <i>et al.</i> 1967
	PPLO-4387, ATCC-14027, human	28.4	B	McGee <i>et al.</i> 1967
<i>M. canis</i>	PG-14, canine	28.4	B	McGee <i>et al.</i> 1967
<i>M. spumans</i>	PG-13, canine	28.3	B	McGee <i>et al.</i> 1967
<i>Mycoplasma</i> sp.	NEGRONI, human	28.0	B	McGee <i>et al.</i> 1967
<i>M. gallinarum</i>	ATCC-15319, avian	27.8	B	Rogul <i>et al.</i> 1965
	PG-16, ATCC-15319, avian	27.5	B	McGee <i>et al.</i> 1967
<i>Mycoplasma</i> sp.	GDL	27.8	B	McGee <i>et al.</i> 1967
<i>M. fermentans</i>	GII, ATCC-15474, human	27.8	B	McGee <i>et al.</i> 1967
	G, human	27.6	C	Neimark, 1967
<i>M. pulmonis</i>	KON, ATCC-14267, human	27.5	B	McGee <i>et al.</i> 1967
<i>M. hyorhinis</i>	BTS-7, ATCC-17981, porcine	27.3	B	McGee <i>et al.</i> 1967
<i>M. pharyngis</i>	LGM, ATCC-19524, human	27.0	B	McGee <i>et al.</i> 1967
<i>M. maculosum</i>	PG-15, canine	26.7	B	McGee <i>et al.</i> 1967
<i>M. orale</i> , type 1	823 B, ATCC-15539, human	26.6	B	McGee <i>et al.</i> 1967
<i>M. orale</i> , type 2	DC-1600, human	26.4	B	McGee <i>et al.</i> 1967
<i>M. mycoides</i> var. <i>mycoides</i>	bovine	26.8, 26.5	B, C	Neimark, 1967
	v 5, bovine	26.1	B	McGee <i>et al.</i> 1967
<i>Mycoplasma</i> sp.	Calf	23.6, 26.5	B, C	Neimark, 1967
	Calf	23.5-26.5	B, C	Neimark & Pene, 1965
<i>M. mycoides</i> var. <i>capri</i>	PG-3, goat	24-26	A	Jones & Walker, 1963
				Jones <i>et al.</i> 1965
<i>Mycoplasma</i> sp.	Kid strain, goat	24.1, 25.5	B, C	Neimark, 1967
	Kid	24.0-25.5	B, C	Neimark & Pene, 1965
<i>M. salivarium</i>	MANIRE, human	23.3	B	McGee <i>et al.</i> 1967
<i>M. neurolyticum</i>	KSA, ATCC-15049, mouse	23.0	B	McGee <i>et al.</i> 1967
<i>M. neurolyticum</i>	Type A, mouse	22.8	C	Neimark, 1967

* A, by hydrolysis and chromatography; B, by thermal denaturation-absorbance measurement; C, by CsCl density gradient centrifugation.

The reports in the literature (Table 3) indicate a range of values for the *Mycoplasma* group from 22.8 to 41.0 moles %. *Mycoplasma pneumoniae* sets apart with reported values from 38.6 to 41 % while the others present a continuous gradation from 22.8 % to 35.0 %.

The technical assistance of Mrs Janet Bergendahl is gratefully acknowledged.

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