Bovine Mycoplasmas: Genome Size and Base Composition of DNA

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SUMMARY

Within the order Mycoplasmatales guanine plus cytosine contents and genome sizes have been determined for 17 strains representing 15 bovine and two ovine species, subspecies or serogroups. The guanine plus cytosine contents were found to vary between 24.4 and 32.9 %. The genome sizes were 4.0 to 5.6×10^8 daltons for 14 sterol-requiring strains and 0.99 to 1.1×10^9 daltons for three non-sterol-requiring strains. These results support earlier findings that members of the genus *Acholeplasma* (family Acholeplasmataceae) have genome sizes about twice those of the genus *Mycoplasma* (family Mycoplasmataceae).

INTRODUCTION

Determination of the mean guanine plus cytosine (GC) contents of mycoplasmal DNA has shown that mycoplasmas form a rather heterogeneous group with variations from 23 to 40 % (Neimark, 1970). Studies by Bak, Black, Christiansen & Freundt (1969) suggest that the sterol-requiring genus *Mycoplasma* (family Mycoplasmataceae) and the non-sterol-requiring genus *Acholeplasma* (family Acholeplasmataceae, Edward & Freundt, 1970) are also different in regard to their genome sizes. The *Mycoplasma* species tested have genome sizes within the range of 4 to 5×10^8 daltons, whereas those of the *Acholeplasma* species are about 1.0×10^9 daltons.

The purpose of this work was to determine the GC contents and the genome sizes of type or reference strains of all known mycoplasma species, subspecies and serogroups of bovine source. The type strains (PG2 and PG3) of two ovine organisms (*Mycoplasma agalactiae* subsp. *agalactiae* and *M. mycoides* subsp. *capri*) were included in the study because of serological relatedness to the bovine strains (*M. agalactiae* subsp. *bovis* (Donetta) and *M. mycoides* subsp. *mycoides* (PG1), respectively).

METHODS

The mycoplasmas investigated (Table 1) were obtained from Drs D. G. FF. Edward, J. Fabricant and E. A. Freundt and from the National Collection of Type Cultures, Colindale, London (Ernø & Stipkovits, 1973).

The substrates used for cultivation were in most cases Bacto Heart Infusion Broth (Difco), supplemented with 15 % horse serum or 1 % PPLO Serum Fraction, 8 % fresh yeast extract, 0.003 % phenol red, 0.08 % thallium acetate and penicillin (400 i.u./ml). Some strains were also supplied with 0.8 % glucose (PG49, PG50, B107PA, PG1, PG3) or 1 % arginine (B139P, B142P, G230).

Mycoplasma dispar was cultivated in FF II medium (Friis, 1972), with minor modifications; it comprised Bacto Brain Heart Infusion Broth (Difco, Detroit, Michigan, U.S.A.),

Table 1. *Melting temperatures, base compositions and genome sizes of DNA from fifteen bovine and two ovine mycoplasma species*

Species or serogroup	Type or reference strain	Melting temperature* (°C)	Base composition (% GC)	Genome size* (10 ⁶ daltons)
<i>Mycoplasma dispar</i>	462/2	81.3 ± 0.05 (4)	29.3	5.3 ± 0.4 (3)
<i>M. bovirhinis</i>	PG 43	80.5 ± 0.15 (6)	27.3	4.4 ± 0.2 (3)
<i>M. mycoides</i> subsp. <i>mycoides</i>	PG1	80.4 ± 0.10 (9)	27.1	5.0 ± 0.1 (3)
<i>M. mycoides</i> subsp. <i>capri</i>	PG 3	79.3 ± 0.00 (4)	24.4	5.0 ± 0.3 (5)
Group L (Al-Aubaidi)	B144P	79.7 ± 0.15 (6)	25.4	4.9 ± 0.4 (5)
Group 7 (Leach)	PG50	79.7 ± 0.05 (4)	25.4	5.6 ± 0.2 (6)
<i>M. bovigenitalium</i>	PG11	81.7 ± 0.05 (4)	30.2	4.0 ± 0.3 (4)
<i>M. agalactiae</i> subsp. <i>agalactiae</i>	PG 2	81.8 ± 0.10 (5)	30.5	4.7 ± 0.3 (3)
<i>M. agalactiae</i> subsp. <i>bovis</i>	Donetta	80.7 ± 0.05 (7)	27.8	4.4 ± 0.1 (2)
<i>M. arginini</i>	G230	80.6 ± 0.15 (4)	27.6	4.0 ± 0.3 (6)
Group 8 (Leach)	PG51	79.9 ± 0.10 (4)	25.9	4.9 ± 0.3 (5)
Group H (Al-Aubaidi)	B139P	79.8 ± 0.10 (5)	25.6	4.4 ± 0.4 (3)
Group I (Al-Aubaidi)	B142P	80.6 ± 0.05 (4)	27.6	4.8 ± 0.1 (2)
Unclassified	M165/69†	81.2 ± 0.10 (2)	29.0	4.3 ± 0.3 (2)
<i>Acholeplasma laidlawii</i>	PG8	82.8 ± 0.20 (12)	32.9	11.0‡
Group 6 (Leach)	PG49	81.3 ± 0.15 (5)	29.3	9.9 ± 1.3 (4)
Group K (Al-Aubaidi)	B107PA	81.4 ± 0.10 (4)	29.5	9.9 ± 0.8 (5)

* Measured values and standard deviations. Number of determinations are given in parentheses.

† Isolated by Langford & Dorward (1969).

‡ Previously reported (Bak *et al.* 1969).

10 % horse serum, 10 % SPF pig serum, 0.05 % yeast extract (Difco), 1 % fresh yeast extract, 0.125 % lactalbumine hydrolysate (Difco), 0.5 % Bacto PPLO broth (Difco) and 0.1 % glucose in Hanks's balanced salt solution and double-distilled water. Phenol red (0.002 %), thallium acetate (0.009 %) and bacitracin (0.02 %) were also added.

Cultures were obtained by inoculation of 50 ml of broth with 0.1 ml stock culture (derived from a single colony, containing about 10⁸ organisms/ml and stored at -70 °C). After incubation at 37 °C for at least 24 h 5000 ml of substrate were inoculated with this pre-culture and harvested in the late log phase in the Sorvall continuous flow system at 48000 g, flow rate 20 ml/min. The DNA was isolated according to Marmur (1961) with the modifications described by Bak & Stenderup (1969). Melting-point determinations were made by measuring extinction of DNA in 1 × SSC (0.15 M-NaCl and 0.015 M-trisodium citrate) under controlled temperature increase (0.25 °C/min) in a Unicam SP800 spectrophotometer equipped with an SP 876 temperature programme controller and an X-Y recorder. The calculation of the GC content from the melting temperature was done according to Marmur & Doty (1962).

The genome size determinations were performed by the renaturation method of Wetmur & Davidson (1968) with the modifications described by Bak *et al.* (1969) and Bak, Christiansen & Stenderup (1970).

RESULTS

Melting temperatures and base compositions are shown in Table 1. The GC contents are found to be between 24.4 and 32.9 %, which is within the range known for mycoplasmal DNA.

Fourteen calculated genome sizes (Table 1) vary between 4.0 and 5.6×10^8 daltons. The deoxyribonucleic acids from the three remaining strains representing *Acholeplasma laidlawii*, serogroup 6 of Leach, and serogroup K of Al-Aubaidi have genome sizes about twice as large (0.99 to 1.1×10^9 daltons). It should be mentioned that the genome size for *A. laidlawii* is the value determined by Bak *et al.* (1969).

DISCUSSION

GC contents of nine of the mycoplasmas studied have been reported previously. Regarding six of these species or subspecies (*Mycoplasma dispar*, *M. mycoides* subsp. *mycoides*, *M. mycoides* subsp. *capri*, *M. bovis genitalium*, *M. arginini* and *Acholeplasma laidlawii*) our results are in good agreement with earlier investigations (Neimark & Pené, 1965; McGee, Rogul & Wittler, 1967; Morowitz, Bode & Kirk, 1967; Neimark, 1967; Chelton, Jones & Walker, 1968; Kelton & Mandel, 1969; Williams, Wittler & Burris, 1969; Gourlay & Leach, 1970; Neimark, 1970). Discrepancies were, however, found as regards *Mycoplasma bovirhinis* and both subspecies of *M. agalactiae*. Using the method of thermal denaturation, Neimark (1967) gave the GC content of 33.6 % for a strain of *M. agalactiae* and Morowitz *et al.* (1967) found the melting temperature of the DNA of *M. agalactiae* subsp. *bovis* (strain Donetta) to be 82.8°C corresponding to a GC content of 32.9 %. In these cases our results are 3 and 5 % lower. With regard to *M. bovirhinis* our result is 2 % higher than the GC content (25.4 %) given by Williams *et al.* (1969).

Our results do not add further to the current discussion whether PG1 and PG3 should be regarded as variants of the same species, *Mycoplasma mycoides*, or representatives of two distinct species (Edward & Freundt, 1969). Although their GC contents differ by approximately 3 % this difference does not exclude a high degree of homology between the deoxyribonucleic acids. Similar considerations apply to PG2 and Donetta, the type strains of the two subspecies of *M. agalactiae*.

Electron microscopy of *Mycoplasma agalactiae* subsp. *bovis* indicates a genome size of 5.9×10^8 daltons (Morowitz *et al.* 1967), which agrees closely with our determination.

PG49 (serogroup 6 of Leach) and B107PA (serogroup K of Al-Aubaidi) as well as the type strain of *Acholeplasma laidlawii* (PG8) were also found to have a genome size characteristic of the Acholeplasmataceae. Only these three strains have been found to be non-sterol-requiring and sensitive to digitonin (Edward, 1971; Ernø & Stipkovits, in preparation, so they belong to Acholeplasmataceae. Our results confirm the findings of Bak *et al.* (1969) that the Acholeplasmataceae have genome sizes in the range of 1×10^9 daltons, whereas Mycoplasmataceae have genome sizes of about half this size (4.0 to 5.6×10^8 daltons).

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