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 x 10⁹ daltons for 14 sterol-requiring strains and 0·99 to 1·1 x 10⁹ 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 x 10⁹ daltons, whereas those of the Acholeplasma species are about 1·0 x 10⁹ 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 I) 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

<table>
<thead>
<tr>
<th>Species or serogroup</th>
<th>Type or reference strain</th>
<th>Melting temperature* (°C)</th>
<th>Base composition (°C, GC)</th>
<th>Genome size* (10^8 daltons)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycoplasma dispar</em></td>
<td>462/2</td>
<td>81.3 ± 0.05 (4)</td>
<td>29.3</td>
<td>5.3 ± 0.4 (3)</td>
</tr>
<tr>
<td><em>M. bovirhinis</em></td>
<td>PG43</td>
<td>80.5 ± 0.15 (6)</td>
<td>27.8</td>
<td>4.4 ± 0.2 (3)</td>
</tr>
<tr>
<td><em>M. mycoides subsp. mycoides</em></td>
<td>PG1</td>
<td>80.4 ± 0.10 (9)</td>
<td>27.1</td>
<td>5.0 ± 0.1 (3)</td>
</tr>
<tr>
<td><em>M. mycoides subsp. capri</em></td>
<td>PG3</td>
<td>79.3 ± 0.00 (4)</td>
<td>24.4</td>
<td>5.0 ± 0.3 (5)</td>
</tr>
<tr>
<td>Group L (Al-Aubaidi)</td>
<td>B144P</td>
<td>79.7 ± 0.15 (6)</td>
<td>25.1</td>
<td>4.9 ± 0.4 (5)</td>
</tr>
<tr>
<td>Group 7 (Leach)</td>
<td>PG50</td>
<td>79.7 ± 0.05 (4)</td>
<td>25.4</td>
<td>5.6 ± 0.2 (6)</td>
</tr>
<tr>
<td><em>M. bovigenitalium</em></td>
<td>PG11</td>
<td>81.7 ± 0.05 (4)</td>
<td>30.2</td>
<td>4.0 ± 0.3 (4)</td>
</tr>
<tr>
<td><em>M. agalactiae</em> subsp. agalactiae</td>
<td>PG2</td>
<td>81.8 ± 0.10 (5)</td>
<td>30.5</td>
<td>4.7 ± 0.3 (3)</td>
</tr>
<tr>
<td><em>M. agalactiae</em> subsp. bovis</td>
<td>Donetta</td>
<td>80.7 ± 0.05 (7)</td>
<td>27.8</td>
<td>4.4 ± 0.1 (2)</td>
</tr>
<tr>
<td>M. arginini</td>
<td>G230</td>
<td>80.6 ± 0.15 (4)</td>
<td>27.6</td>
<td>4.0 ± 0.3 (6)</td>
</tr>
<tr>
<td>Group 8 (Leach)</td>
<td>PG51</td>
<td>79.9 ± 0.10 (4)</td>
<td>25.9</td>
<td>4.9 ± 0.3 (5)</td>
</tr>
<tr>
<td>Group H (Al-Aubaidi)</td>
<td>B139P</td>
<td>79.8 ± 0.10 (5)</td>
<td>25.6</td>
<td>4.4 ± 0.4 (3)</td>
</tr>
<tr>
<td>Group I (Al-Aubaidi)</td>
<td>B142P</td>
<td>80.6 ± 0.05 (4)</td>
<td>27.6</td>
<td>4.8 ± 0.1 (2)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>M165/69†</td>
<td>81.2 ± 0.10 (2)</td>
<td>29.0</td>
<td>4.3 ± 0.3 (2)</td>
</tr>
<tr>
<td><em>Acholeplasma laidlawii</em></td>
<td>PG8</td>
<td>82.8 ± 0.20 (12)</td>
<td>32.9</td>
<td>11.0±2</td>
</tr>
<tr>
<td>Group 6 (Leach)</td>
<td>PG49</td>
<td>81.3 ± 0.15 (5)</td>
<td>29.3</td>
<td>9.9 ± 1.3 (4)</td>
</tr>
<tr>
<td>Group K (Al-Aubaidi)</td>
<td>B107PA</td>
<td>81.4 ± 0.10 (4)</td>
<td>29.5</td>
<td>9.9 ± 0.8 (5)</td>
</tr>
</tbody>
</table>

* Measured values and standard deviations. Number of determinations are given in parentheses.
† Previously reported by Langford & Dorward (1969).
†† Previously reported by Bak et al. (1969).

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 \(5.6 \times 10^8\) 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 \(1.1 \times 10^9\) 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. bovigenitalium, 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 \(5.9 \times 10^8\) 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 \(1 \times 10^9\) daltons, whereas Mycoplasmataceae have genome sizes of about half this size (\(4.0\) to \(5.6 \times 10^8\) daltons).

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