Deoxyribonucleic Acid Base Composition of Lactobacilli Determined by Thermal Denaturation

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Moles per cent guanine plus cytosine content of 16 lactobacilli provided three taxonomic groups: I, 32.4 to 38.3% with five species; II, 42.7 to 48.0% with six species; III, 49.0 to 51.9% with five species.

Gasser and Mandel (10) compared the results of buoyant density determinations of deoxyribonucleic acid (DNA) isolated from various lactobacilli with results previously obtained by chemical analysis (11) and by thermal denaturation (Tm; 1–5). They established a definite linearity between the buoyant density of DNA in CsCl and the guanine plus cytosine (GC) content determined by chemical methods. However, when GC values derived from buoyant density measurements were compared with those determined by thermal denaturation, several significant variations were observed. The results presented herein complement and extend the literature available on the DNA base composition of lactobacilli and further clarify the variations observed by using different methodologies.

Species of lactobacilli were obtained from the stock culture collection of the Department of Microbiology, Oregon State University, and from M. Elizabeth Sharpe, National Institute for Research in Dairying, University of Reading, England. Sporolactobacillus inulinus was received from K. Kitahara and J. Suzuki, Tokyo University of Agriculture, Setagaya-ku, Tokyo, Japan. All cultures were reevaluated as to species designation by accepted procedures (16–18). Lactobacilli were grown in MRS-medium (7) at their optimal growth temperature, 32 or 37 C; S. inulinus was grown in lactic broth (8) at 37 C. Lactobacillus helveticus and S. inulinus were susceptible to lysis by the addition of lysozyme and then sodium lauryl sulfate. The remaining lactobacilli were lysed by using a dual enzyme system containing lysozyme and lytase (BBL). When necessary, cellular suspensions were heated at 70 C for 30 to 60 min and cooled to room temperature prior to the addition of the above enzymes. The rest of the extraction procedure was by the method of Marmur (13); however, subtilisin (20 μg/ml) was added to remove ribonuclease. Tmax values of purified DNA extracts were determined with a Gilford (Model 2000) automatic spectrophotometer. The Tmax values recorded were calculated as described by Marmur and Doty (14) and by normal probability plots of melting data (12). Moles % GC was calculated according to the linear relation of Marmur and Doty (14).

All DNA preparations gave monophasic absorption-temperature denaturation profiles, and the compositional distribution of DNA molecules was relatively narrow and unimodal. The Tmax values reported are an average of at least two determinations and were reproducible for a given sample within 0.3 C; the Tmax values for different DNA preparations of the same culture were also reproducible within 0.3 C. Tmax and moles % GC values of recognized species of lactobacilli are given in Table 1. The Tmax values calculated as described by Marmur and Doty (14) are in close agreement with those determined by normal probability plots of melting data. Since DNA base compositions ranged from 32.4 to 51.9% GC, heterogeneity within the genus Lactobacillus is quite evident. This observation is not unique for the lactobacilli because compositional heterogeneity has also been reported within other genera: Proteus, 39 to 50% (9); Neisseria, 40 to 50% (6, 14); Mycoplasma, 24 to 41% (15, 20); and Bacillus, 33 to 50% (14).

Comparisons of data from this study with compiled GC contents for lactobacilli from previous reports (1–5, 10) are shown in Table 2. Our observations do not concur entirely with those
reported by Cantoni and co-workers. Significant differences were noted for L. leichmannii, L. delbrueckii, and L. lactis. Additional variations were observed for L. helveticus, L. jugurti, L. cellobiosus, and L. salivarius. With the exception of L. acidophilus and L. bulgaricus, our data substantiate results obtained by buoyant density measurements (Table 2) as well as by chemical analyses (11). The variations observed between our data and that previously reported by Cantoni and associates may be from the use of different strains, improper species designation, or inaccuracies in the physical determinations.

On the basis of GC content, the lactobacilli were placed in three groups similar to those

Table 1. Moles per cent guanine plus cytosine content (mole % GC) and thermal denaturation (Tm) values of lactobacilli

<table>
<thead>
<tr>
<th>Organisms and origina</th>
<th>Tm (°C)</th>
<th>Tm (°C) (NPP)b</th>
<th>Moles % GCc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus helveticus OSU</td>
<td>85.0</td>
<td>85.0</td>
<td>38.3</td>
</tr>
<tr>
<td>L. jugurti ATCC 521</td>
<td>84.5</td>
<td>84.5</td>
<td>37.1</td>
</tr>
<tr>
<td>L. plantarum P5 (NCDO 343)</td>
<td>87.3</td>
<td>87.4</td>
<td>43.9</td>
</tr>
<tr>
<td>L. plantarum 17-5 (ATCC 8014)</td>
<td>87.8</td>
<td>87.8</td>
<td>45.1</td>
</tr>
<tr>
<td>L. (jugurti) bulgaricus ATCC 7993</td>
<td>84.6</td>
<td>84.5</td>
<td>37.3</td>
</tr>
<tr>
<td>L. acidophilus Farr</td>
<td>89.9</td>
<td>89.7</td>
<td>50.2</td>
</tr>
<tr>
<td>L. lactis 39-A</td>
<td>89.4</td>
<td>89.3</td>
<td>49.0</td>
</tr>
<tr>
<td>L. casei ATCC 9595</td>
<td>88.1</td>
<td>88.2</td>
<td>45.8</td>
</tr>
<tr>
<td>L. casei ATCC 7469 (NCDO 243)</td>
<td>88.7</td>
<td>88.5</td>
<td>47.3</td>
</tr>
<tr>
<td>L. casei C5 (NCDO 151)</td>
<td>87.8</td>
<td>88.7</td>
<td>47.3</td>
</tr>
<tr>
<td>L. casei 780</td>
<td>88.9</td>
<td>89.0</td>
<td>47.8</td>
</tr>
<tr>
<td>L. casei 356</td>
<td>88.8</td>
<td>88.8</td>
<td>47.5</td>
</tr>
<tr>
<td>L. casei 300</td>
<td>88.9</td>
<td>88.8</td>
<td>47.8</td>
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<tr>
<td>L. casei 316</td>
<td>89.0</td>
<td>88.9</td>
<td>48.0</td>
</tr>
<tr>
<td>L. brevis X1 (NCDO 473)</td>
<td>87.8</td>
<td>87.9</td>
<td>45.1</td>
</tr>
<tr>
<td>L. salivarius ATCC 11742</td>
<td>82.6</td>
<td>82.3</td>
<td>32.4</td>
</tr>
<tr>
<td>L. viridescens ATCC 12706</td>
<td>86.8</td>
<td>86.8</td>
<td>42.7</td>
</tr>
<tr>
<td>L. bulgaricus ATCC 12278 (GA)</td>
<td>85.0</td>
<td>84.8</td>
<td>38.3</td>
</tr>
<tr>
<td>L. buchneri BC1 (NCDO 110)</td>
<td>87.6</td>
<td>87.8</td>
<td>44.6</td>
</tr>
<tr>
<td>L. fermenti F1 (NCDO 215)</td>
<td>90.6</td>
<td>90.4</td>
<td>51.9</td>
</tr>
<tr>
<td>L. cellobiosus G1 (NCDO 927)</td>
<td>90.3</td>
<td>90.1</td>
<td>51.2</td>
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<tr>
<td>L. leichmannii ATCC 7830</td>
<td>90.0</td>
<td>90.0</td>
<td>50.5</td>
</tr>
<tr>
<td>L. leichmannii ATCC 4797</td>
<td>90.0</td>
<td>90.1</td>
<td>50.5</td>
</tr>
<tr>
<td>L. delbrueckii ATCC 9649</td>
<td>89.8</td>
<td>89.5</td>
<td>50.0</td>
</tr>
</tbody>
</table>

- ATCC, American Type Culture Collection; NCDO, National Collection of Dairy Organisms, Reading, England.
- Tm values were obtained from normal probability plots of melting data.
- Moles % GC content were calculated from Tm values in column two.

Table 2. Comparison of reported moles per cent guanine plus cytosine (moles % GC) of various lactobacilli

<table>
<thead>
<tr>
<th>Organism</th>
<th>Moles % GC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thermal denaturationa</td>
</tr>
<tr>
<td>Lactobacillus helveticus</td>
<td>38.3</td>
</tr>
<tr>
<td>L. jugurti</td>
<td>37.1</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>43.9</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>50.2</td>
</tr>
<tr>
<td>L. lactis</td>
<td>49.0</td>
</tr>
<tr>
<td>L. casei</td>
<td>47.4</td>
</tr>
<tr>
<td>L. brevis</td>
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</tr>
<tr>
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<td>32.4</td>
</tr>
<tr>
<td>L. viridescens</td>
<td>42.7</td>
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<tr>
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<tr>
<td>L. fermenti</td>
<td>51.9</td>
</tr>
<tr>
<td>L. cellobiosus</td>
<td>51.2</td>
</tr>
<tr>
<td>L. leichmannii</td>
<td>50.5</td>
</tr>
<tr>
<td>L. delbrueckii</td>
<td>50.0</td>
</tr>
</tbody>
</table>

- Data from this study.
- Data previously reported in the literature (1-5) and compiled by Gasser and Mandel (10).
- From Gasser and Mandel (10).
- Mean GC content for eight strains.

proposed by Gasser and Sebald (11) and Gasser and Mandel (10). Group I includes species with a GC content between 32.4 and 38.3%: L. iugurti, L. helveticus, L. salivarius, L. (jugurti) bulgaricus and L. bulgaricus GA. Gasser and Sebald (11) also included L. acidophilus; however, our strain, L. acidophilus Farr, had a GC content of 50.2%. Since L. acidophilus Farr and L. lactis 39-A gave identical fermentation reactions and have similar GC contents (Table 1), strain Farr may have been classified erroneously and should be redesignated L. lactis. Group II includes species with a GC content between 42.7 and 48.0%: L. buchneri, L. brevis, L. casei, L. viridescens, and L. plantarum. Group III includes species with a GC content between 49.0 and 51.9%: L. lactis, L. leichmannii, L. delbrueckii, L. fermenti and L. cellobiosus. Further distinctions within each of the above groups may become apparent when the results of DNA-RNA (ribo-
nucleic acid) hybridization experiments, now in progress, are evaluated

*S. inulinus* DNA was also analyzed and found to have a GC content of 47.3%. Suzuki and Kitahara (19) previously reported a value of approximately 39% for the same strain and suggested a possible relationship between *L. leichmannii* and *S. inulinus*.

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**LITERATURE CITED**


