Deoxyribonucleic Acid Base Composition of Some Members of the Subgenera Betabacterium and Streptobacterium

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Abstract

CANTONI, C. (Istituto "P. Stazia," Universita Statale, Milan, Italy), L. R. HILL, and L. G. SILVESTRI. Deoxyribonucleic acid base composition of some members of the subgenera Betabacterium and Streptobacterium. Appl. Microbiol. 13:631–633. 1965.—The base composition of deoxyribonucleic acid (DNA) prepared from four Betabacterium strains and four Streptobacterium strains was determined. Per cent GC values (guanine + cytosine/total bases) of the DNA were evaluated from the "melting-temperatures" (Tm) of the nucleic acids. For the Betabacterium strains, these values ranged from 44 to 51.5% GC, and those for the Streptobacterium strains ranged from 43 to 47.5% GC. The taxonomic division into these two subgenera is not, therefore, supported by these findings.

Analysis of overall similarity and analysis of deoxyribonucleic acid (DNA) base composition represent the most recent techniques applied to microbial systematics. Results obtained with the second of these two approaches were recently reviewed by Marmur, Falkow, and Mandel (1963). Given the economic importance of lactobacilli and the unsatisfactory state of their classification, it is rather surprising to find that data regarding DNA base composition of these bacteria are scant. Only two Lactobacillus species appear to have been studied, and, moreover, these species have very different per cent GC values for their DNA (DNA base composition is conveniently expressed as per cent GC values; guanine + cytosine/total bases): L. acidophilus, 38 to 40% GC; and L. bifidus, 56 to 58% GC (Marmur et al., 1963).

It is well known that classifications of lactobacilli currently accepted (Orla-Jensen, 1919; Sharpe, 1961; Bergey's Manual) show considerable differences and that, in practice, it is almost impossible to use them to satisfactorily identify fresh isolates. In this situation, then, we proceeded to examine the DNA base composition of some deposited strains. Our objectives were (i) to obtain some points of reference for future studies and for eventual divisions into more homogeneous groups than the present ones, and (ii) to investigate the validity of the division into the two subgenera Betabacterium and Streptobacterium.

Materials and Methods

Organisms. The strains of lactobacilli examined belong to the subgenera Betabacterium and Streptobacterium, according to the classification of Orla-Jensen (1919). According to the classification proposed in Bergey's Manual, they belong to the family Lactobacillaceae, tribe Lactobacillae, genus Lactobacillus. In common with most European authors, we adhere to the classification of Sharpe (1961), derived from that of Orla-Jensen. The strains used in the present work, received as lyophilized cultures by courtesy of E. Sharpe, are listed in Table 1; all are strains deposited in the National Collection of Dairy Organisms, Reading, England.

Cultivation and DNA extraction. The organisms were subcultured into M.R.S. broth (Sharpe, 1961) in 500-ml volumes contained in 3-liter Erlenmeyer flasks. After 18 hr of incubation at 30°C, cells were collected by centrifugation. Lysozyme followed by sodium lauryl sulfate was used to lyse the cells. This technique and the subsequent purification of DNA from cell lysates were described by Marmur (1961). Purified DNA was dissolved in 0.1 X SSC (SSC = 0.15 m NaCl + 0.015 m sodium citrate). Tm and per cent GC determinations. Samples of DNA solutions at concentrations of about 10 \( \mu g/\text{ml} \) were heated in hermetically closed cuvettes in a Beckman DU spectrophotometer fitted with thermospacers, as described by Marmur and Doty (1961) and De Ley and Schell (1963), and optical density readings (at 260 m\( \lambda \)) were taken at intervals. Tm ("melting-temperature") is defined as the temperature corresponding to the mid-point of the percentage increase in optical density that occurs during thermal denaturation of the DNA.
TABLE 1. Lactobacillus strains used with Tm
and per cent GC values of their DNA

<table>
<thead>
<tr>
<th>Organism</th>
<th>Reference no.</th>
<th>Tm*</th>
<th>Per cent GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subgenus Streptobacterium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. casei var. rhamnosus</td>
<td>243</td>
<td>73.2</td>
<td>47</td>
</tr>
<tr>
<td>L. casei var. casei</td>
<td>151</td>
<td>72.75</td>
<td>46</td>
</tr>
<tr>
<td>L. casei var. alactosus</td>
<td>680</td>
<td>73.3</td>
<td>47.5</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>343</td>
<td>71.4</td>
<td>43</td>
</tr>
<tr>
<td>Subgenus Betabacterum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. brevis</td>
<td>473</td>
<td>72.4</td>
<td>45.5</td>
</tr>
<tr>
<td>L. fermenti</td>
<td>215</td>
<td>75.0</td>
<td>51.5</td>
</tr>
<tr>
<td>L. buchneri</td>
<td>110</td>
<td>71.75</td>
<td>44</td>
</tr>
<tr>
<td>L. cellobiosus</td>
<td>927</td>
<td>72.5</td>
<td>45.5</td>
</tr>
</tbody>
</table>

* In 0.015 M NaCl + 0.0015 M sodium citrate.
† Per cent GC = (Tm + 15.4) - 69.3/0.41 (Silvestri and Hill, 1965).

Tm values are linearly related to per cent GC values, and, in the present work, the latter were calculated as described by Silvestri and Hill (1965).

RESULTS AND DISCUSSION

Tm values and per cent GC values of the eight DNA preparations examined are listed in Table 1. Base compositions of Streptobacterium strains ranged from 43 to 47.5% GC. However, the three L. casei strains had very similar values (46 to 47.5% GC), whereas L. plantarum was appreciably different (43% GC). Cummins and Harris (1956) found that this homofermentative species of Lactobacillus was different from all other Lactobacillus species they studied, because diamino-pimelic acid was present and aspartic acid was absent in cell-wall hydrolysates. Its DNA has the lowest percentage of GC found among the strains examined here (see Table 1).

Values for Betabacterium strains showed a wider range, from 44 to 51.5% GC, thus overlapping the values for L. casei, L. brevis and L. cellobiosus have the same value (45.5% GC), near to which is that of L. buchnerii (44% GC). Again, the value for L. fermenti is considerably different (51.5% GC).

The results indicate variability in DNA base composition within both subgenera and, moreover, the degree of variability within either of them is almost of the same order as that considering all the strains together. Within the streptobacteria, a fairly homogeneous group, with respect to base compositions, can be recognized, comprising L. casei and its variants. Within the betabacteria, L. brevis and L. cellobiosus might represent another such group.

Though the present results refer to only a limited number of strains, they indicate that a distinction, on the basis of base compositions, between the two subgenera recognized in traditional classifications cannot be made. This finding seems to us to be important, for it may account for the difficulty encountered in identifying fresh isolates with one or the other of the two subgenera. The division rests on the products of glucose fermentation. Betabacterium produces CO₂, whereas Streptobacterium does not. In view of the present results, our opinion is that the monothetic division (Sneath, 1957) yields artificial groups.

Both the previously reported per cent GC values for lactobacilli differ considerably from the present findings. L. bifidus (56 to 58% GC), however, was not considered by Sharpe (1961) to belong to the genus Lactobacillus, and Davis (1964) further suggested it to be an intermediate between lactobacilli and corynebacteria. Per cent GC values for the latter genus range from 48 to 58.5% (Bouisset, Breuillard, and Michel, 1963). The other reported species, L. acidophilus (38 to 40% GC; Sharpe, 1961), is considered not to belong to the subgenus Thermobacterium. Again, it is interesting to note that Davis (1964) considers L. plantarum to be the most primitive form of bacitracin and to possibly represent a phylogenetic link with Thermobacterium.

The finding of considerable variability in DNA base composition among the relatively few strains examined here, and the differences noted with previously reported compositions, suggest that this approach to Lactobacillus taxonomy is worth investigating more thoroughly and that it will probably yield fruitful results.

ACKNOWLEDGMENTS

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LITERATURE CITED


Cummins, C. S., and H. Harris. 1956. The chemical composition of the cell-wall in some gram-


ERRATA

In Vitro and In Vivo Laboratory Evaluation of Cephaloglycin and Cephaloridine

WARREN E. WICK and WILLIAM S. BONIECE
The Lilly Research Laboratories, Indianapolis, Indiana

Volume 13, no. 2, p. 249: the structure printed for cephaloglycin was in error; the correct structure is that shown below.

```
H O
\[\text{C-C-NH\_NH}_2\]
\[\text{O}\]
\[\text{CH}_2\text{OCO}\]
\[\text{CH}_3\]
\[\text{C-OH}\]

Cephaloglycin
```

Pouch Method for the Isolation and Enumeration of Clostridia

B. O. BLADEL and RICHARD A. GREENBERG
Swift and Co. Research and Development Center, Chicago, Illinois

Volume 13, no. 2, p. 281, col. 1, line 3 of “Materials and Methods”: change “per in.$^2$ per 24 hr” to “per 100 in.$^2$ per 24 hr.”