Evidence for Extrachromosomal Elements in Lactobacillus

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Three strains of lactobacilli, Lactobacillus casei subsp. casei 64H, L. casei subsp. rhamnosus OC91, and L. coryniformis M34, were examined for the presence of plasmids. Plasmids of molecular weights of $23 \times 10^6$ and $19 \times 10^6$ were found in the first two strains, respectively. This represents the first evidence for plasmids in lactobacilli; their function is not presently known.

This report describes the isolation of covalently closed-circular (CCC) and open-circular deoxyribonucleic acid (DNA) from Lactobacillus casei subsp. casei and L. casei subsp. rhamnosus. We have used a new technique for preparing and lysing spheroplasts, as well as for isolating CCC DNA (2, 6; T. C. Currier and E. W. Nester, manuscript in preparation).

To our knowledge, the presence of extrachromosomal elements in lactobacilli has never been reported. The molecular weights of the plasmids found suggest the capacity for the expression of many as yet undetermined plasmid-coded traits in L. casei.

The bacterial strains studied, L. casei subsp. casei 64H, L. casei subsp. rhamnosus OC-91, and L. coryniformis M34, were grown 24 h at 37°C in brain heart infusion supplemented with L-threonine (20 mM), deoxyadenosine (250 μg/ml), and [3H]thymidine (5 μCi/ml) ([3H]thymidine was used for L. coryniformis to improve incorporation efficiency). The growth and preparation of spheroplasts and their subsequent lysis were exactly as described previously by Chassy (2). Sodium dodecyl sulfate-containing lysates were sheared, alkali denatured, neutralized quickly, adjusted to 3% NaCl (wt/vol), extracted with phenol saturated with 3% NaCl in water, extracted with CHCl₃-isoamyl alcohol (24:1, vol/vol), and finally ethanol precipitated exactly according to the technique described by Watson et al. (6) and Currier and Nester (in preparation). This technique removes essentially all linear and open-circular DNA that bands at the phenol-H₂O interface and results in deproteinized, ribonucleic acid-free DNA preparations containing 40 to 95% of the [3H]thymine or [3H]thymidine present as CCC-DNA.

Figure 1. Dye-buoyant density centrifugation of purified DNA from L. casei subsp. casei and L. casei subsp. rhamnosus. Cesium chloride-ethidium bromide gradients were prepared (9.2-ml sample in TES buffer; 9.2 g of CsCl; 0.6 ml of ethidium bromide, 10 mg/ml; in a Beckman polylamellar tube) and centrifuged to equilibrium in a type 50 Ti rotor for 44 h at 44,000 rpm at 20°C. The gradients were collected from the bottom, the refractive indices of fractions were measured, and then the ethidium bromide was removed by the addition of an equal volume of a 50% (vol/vol) suspension of Dowex-50 × 12 (200 to 400 mesh, Na⁺ form in SSC buffer). The data in part A were obtained from L. casei subsp. casei; the data presented in part B were obtained from L. casei subsp. rhamnosus (○) and from a partially "nicked" preparation of ¹⁴C-labeled SV40 DNA (○).
### Table 1. Summary of physical properties of *L. casei* plasmids

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sedimentation constant of open circles (×10^6)</th>
<th>Mol wt a (×10^6)</th>
<th>Sedimentation constant (×10^6)</th>
<th>Mol wt b (×10^6)</th>
<th>Contour length ± SD (μm)</th>
<th>Mol wt ×10^6 ± SD (from length)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. casei</em> subsp. <em>casei</em></td>
<td>34S</td>
<td>24</td>
<td>47.4S</td>
<td>22.2</td>
<td>11.8 ± 0.63</td>
<td>23.1 ± 1.23</td>
</tr>
<tr>
<td><em>L. casei</em> subsp. <em>rhamnosus</em></td>
<td>32S</td>
<td>20</td>
<td>44.5S</td>
<td>19.2</td>
<td>9.78 ± 0.68</td>
<td>19.2 ± 1.33</td>
</tr>
</tbody>
</table>

a From an empirical plot of log molecular weight (mol wt) versus log S for plasmids of known molecular weight (for example, open-circular forms of SV40 and the α, β, and γ plasmids of *S. faecalis*).  

b From S CCC DNA = 0.034 M_0.428 (3).  

c Sample size was 43 molecules of *L. casei* subsp. *casei* and 35 molecules of *L. casei* subsp. *rhamnosus* measured. This gives a probability of less than 0.001 that these plasmids have the same molecular weight.  

d Assuming: 1.96 × 10^6 daltons/μm.

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![Fig. 2. Composite of representative electron micrographs of *L. casei* DNA prepared by the Kleinschmidt technique. The molecules are: (a) supercoil from *L. casei* subsp. *casei*, (b) open circle from *L. casei* subsp. *casei*, (c) supercoil from *L. casei* subsp. *rhamnosus*, and (d) open circles from *L. casei* subsp. *rhamnosus.*](http://jb.asm.org/ on August 27, 2017 by guest)
In Fig. 1A,B the data obtained from dye-buoyant density gradients of *L. casei* subsp. *casei* and *rhamnosus* illustrate the existence of CCC DNA as a heavy peak. As a control, data obtained from a preparation of CCC 14C-labeled DNA and open-circular 14C-labeled DNA from SV40 are superimposed in Fig. 1B. Because no satellite or heavy band could be found in *L. coryniformis*, the data are not shown. In contrast to the two *L. casei* strains, plasmids were not evident in this strain when whole lysates, cleared lysates, or Pronase-treated, cleared lysates were analyzed by dye-buoyant density centrifugation. This strain of *L. coryniformis* appears to be plasmidless.

The CCC DNA-containing fractions from dye-buoyant density gradients were pooled and dialyzed against three changes of TES buffer and subjected to ultracentrifugation in neutral sucrose density gradients (data not shown). The α and β plasmids from *Streptococcus faecalis* ATCC 14508 reported by Clewell et al. were used as 43S (supercoiled) and 28S (supercoiled) markers in these gradients (3). Gradients from *L. casei* subsp. *casei* gave two peaks of 47.4S and 34S, while DNA from *L. casei* subsp. *rhamnosus* sedimented at 44.5S and 32S. Upon aging both preparations for 6 weeks, the 34S and 32S peaks became more prominent (approximately 50% of total DNA), suggesting a relaxation of 47.4S and 44.5S supercoils to open circles of lower S values (Table 1).

Electron microscopy by the Davis modification (4) revealed both supercoiled and open-circular forms in both *L. casei* strains. Representative micrographs are shown in Fig. 2. The average contour length of the open circles found in *L. casei* subsp. *casei* is 11.8 ± 0.63 μm, corresponding to a molecular weight of 23.1 × 10⁶ ± 1.23. The plasmid found in *L. casei* subsp. *rhamnosus* had a contour length of 9.78 ± 0.68 μm and a molecular weight of 19.2 ± 1.33 (see Table 1).

Based on DNA recovery, the yield of plasmid DNA corresponds to a minimum frequency of 3 to 5 plasmid copies per host genome. (The actual genome size of these lactobacilli is unknown, but was assumed to be 2 × 10⁹.)

The *L. casei* strains studied here have been found not to have antibiotic resistance or bacteriocin or hemolysin production, so these may be ruled out as plasmid-coded traits (J. London and D. LeBlanc, unpublished observations). The two organisms studied are, however, human isolates; similar strains have been implicated in the etiology of pit and fissure carious lesions in children (1). In addition, *L. casei* has been shown to cause bacterial endocarditis (5). It is tempting to speculate that surface antigens, epithelial adherence sites, or possibly catabolic enzymes could be coded for by plasmid DNA in these organisms. Experiments directed at testing these possibilities are in progress.

Previously there was no reported evidence of plasmids in lactobacilli. The lysis and isolation procedure used here makes plasmid characterization in lactobacilli a straightforward procedure. Before this report, lactobacilli were thought to be difficult to lyse; our experience indicates that the lysis procedure for streptococci reported by Chassy (2) is applicable to common lactobacilli. This invites studies not only of the plasmid-coded properties of lactobacilli, but of the evolution and relatedness of lactobacilli as a function of plasmid-mediated traits.

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**LITERATURE CITED**


