Plasmid Profiles and Transfer of Plasmid-Encoded Antibiotic Resistance in Lactobacillus plantarum

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Plasmids were visualized in strains of Lactobacillus plantarum by use of a rapid method. Plasmids pIP501 and pAMβ1 were transferred by conjugation from Streptococcus strains to Lactobacillus plantarum, and recipient strains were shown to act as donors in crosses to S. lactis. Attempts to transfer these plasmids between strains of L. plantarum were not successful.

The lactobacilli are a commercially important group of organisms, particularly in the food-processing industry. The occurrence of plasmids in several species of lactobacilli has now been reported (1, 8, 10, 12), and transfer of the antibiotic resistance plasmids pAMβ1 and pIP501 to two species of Lactobacillus (6, 13) has been achieved. Lactobacillus plantarum is important in the production of fermented meats and vegetables, as well as in the fermentation of grass to produce silage. Genetic investigations will allow this species to be optimized for use in its present roles and permit its application to new industrial processes.

Strains used were as follows: L. plantarum NCDO 340, 343, 352, 704, 1193, and 1752; Streptococcus lactis NCDO 712 lac" (pAMβ1); and S. faecalis JH2-1 and JH2-2(pIP501). Plasmids pAMβ1 (2) and pIP501 (5) specify erythromycin resistance; pIP501 also specifies resistance to chloramphenicol.

L. plantarum was grown on MRSB medium (4) and incubated at 37°C. S. lactis and S. faecalis were grown on M17 medium (11) and incubated at 30 and 37°C, respectively. Lactose or glucose (0.5%, wt/vol) was added. Spontaneous antibiotic-resistant mutants of the L. plantarum strains were obtained by plating overnight cultures on media containing streptomycin (1 mg/ml) or rifampin (400 μg/ml).

A rapid screening technique was developed to examine L. plantarum strains for plasmids. Cultures (10 ml; A660, 0.7) were harvested by centrifugation and pellets were suspended in 100 μl of protoplast buffer (0.04 M ammonium acetate, 0.001 M magnesium acetate, 0.5 M sucrose [pH 7]) containing lysozyme (7 mg/ml). Incubation (37°C for 5 min) was followed by addition of diethyl pyrocarbonate (12.5 μl) and a further incubation (37°C for 10 min). Lysis buffer (TE buffer [0.1 M Tris, 0.01 M EDTA [pH 10.5] containing sodium dodecyl sulfate [1%, wt/vol]]) was added, and after mixing, neutralization was achieved by the addition of 2 M Tris (pH 7) followed by 5 M NaCl (400 μl). The mixture was incubated at room temperature (30 min), and then 1 volume of phenol-chloroform-isooamyl alcohol (25:24:1) was added. After mixing and centrifugation (10,000 × g for 20 min at 4°C) the supernatant fluid was removed, 1 volume of chloroform-isooamyl alcohol (24:1) was introduced, and the solution was thoroughly mixed. After incubation on ice (10 min) and centrifugation (10,000 × g for 20 min at 4°C), the aqueous fraction was removed. Precipitation overnight with isopropanol (9) produced a DNA pellet which was resuspended in 10 to 30 μl of TES buffer (0.05 M Tris, 0.005 M EDTA, 0.05 M NaCl [pH 8]), and 2.5 μl of RNase (1 mg/ml in 0.005 M sodium acetate [pH 5]) was added. After incubation (at 37°C for 1 h), the DNA was subjected to agarose gel electrophoresis on 0.7 or 1% (wt/vol) gels. The plasmid profile of S. lactis 712 (3) was used as a standard to estimate the molecular weights of plasmid bands. Covalently closed circular DNA was distinguished from open circular and linear DNA as described previously (7).

This method can be adapted to lyse larger batch cultures (1 liter) to isolate plasmid DNA from cesium chloride gradients. The RNase step and the chloroform-isooamyl alcohol extraction were omitted, and the DNA pellet obtained after precipitation was resuspended in TES buffer (3 ml) and then added to 3.25 g of cesium chloride and 150 μl of ethidium bromide (1.5% [wt/vol] in TES buffer), then centrifuged (120,000 × g for 7 h at 10°C) in a vertical rotor. The plasmid band was collected, extracted with cesium chloride-saturated isopropanol, and dialyzed against TE buffer (2 h), with three changes of buffer.

In filter matings, equal amounts of donor and recipient (overnight cultures) were trapped on a filter (pore size, 0.45 μm), which was then placed on nonselective medium and inoculated overnight. Cells were washed off the filter, serially diluted, and spread on selective media. After incubation, colonies arising on each plate were counted. Controls of donor and recipient cells alone received the same treatment. Transfer was selected with erythromycin (50 μg/ml), and for pIP501, resistance to chloramphenicol (20 μg/ml) was subsequently tested as an unselected marker. The donor was counterselected with either streptomycin (1 mg/ml) or rifampin (400 μg/ml). So that transfer frequencies could be determined, the number of cells per milliliter in each culture used was determined by dilution plating.

A rapid technique for visualizing plasmids in several strains of L. plantarum has been developed. This method is quicker than any previously published and can be readily adopted for use to distinguish between strains on the basis of plasmid content. The plasmid profiles of strains as visualized when the rapid screening procedure was used are shown in Fig. 1, although two plasmid bands in strain NCDO 340 have not been seen reproducibly and are not shown. The number of plasmid bands observed is given in Table 1. Plasmids were observed in all six strains of L. plantarum. To distinguish between plasmids in open circular, covalently closed circular, and linear configurations, agarose gels showing DNA from each strain were irradiated with UV light and then further electrophoresed (Table 1). The techniques described here yield plasmid DNA from L. plantarum strains in a
NOTES

in three large plasmids interpretation of 704; examination by both reported previously (6) has reported publication pIP501 and pAMP1-encoded erythromycin to L. were examined derivatives of other lactic acid bacteria examined pAMP1 be visualized Tyr-1752 (Fig. 1). No. of No. of Frequencies

704 352 10 6 15, 10, 8.5, 5 
340 2 1 11.5 
1752 2 1 7

FIG. 2. Exconjugants of L. plantarum, showing (a) the presence of pIP501, (b) wild type, and (c) the presence of pAMβ1. The 7-kilobase plasmid present in this strain (pCIT100) can also be seen.

TABLE 2. Transfer frequencies of pAMβ1 and pIP501 per recipient

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Recipient strain</th>
<th>Transfer frequency</th>
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<tbody>
<tr>
<td>S. lactis 712 lac-(pAMβ1)</td>
<td>L. plantarum 340</td>
<td>1.1 × 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>L. plantarum 352</td>
<td>3.9 × 10⁻⁶</td>
</tr>
<tr>
<td></td>
<td>L. plantarum 1752</td>
<td>3.9 × 10⁻⁵</td>
</tr>
<tr>
<td>S. faecalis JH2-2(pIP501)</td>
<td>L. plantarum 340</td>
<td>1.2 × 10⁻⁶</td>
</tr>
<tr>
<td></td>
<td>L. plantarum 352</td>
<td>1 × 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>L. plantarum 1752</td>
<td>6.8 × 10⁻⁷</td>
</tr>
<tr>
<td>L. plantarum 1752(pAMβ1)</td>
<td>S. lactis 712</td>
<td>3.6 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>L. plantarum 340</td>
<td>ND</td>
</tr>
<tr>
<td>L. plantarum 352(pAMβ1)</td>
<td>L. plantarum 340</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>L. plantarum 1752</td>
<td>ND</td>
</tr>
<tr>
<td>L. plantarum 1752(pIP501)</td>
<td>L. plantarum 340</td>
<td>ND</td>
</tr>
</tbody>
</table>

*a lac-*, Plasmid-cured strain, unable to utilize lactose.
*ND, None detected.

observed, it was not possible to visualize the plasmid DNA in exconjugants, and it was suggested that this plasmid DNA may have become incorporated into the chromosome. We have found that pAMβ1 can be visualized in exconjugants of L. plantarum as a distinct band, but with only poor resolution and reproducibility, and that pIP501 can be seen only a little more easily. It is therefore possible that the techniques used previously did not allow elucidation of pAMβ1 plasmid DNA. Intragenic transfer between strains of L. plantarum, with donors to which these antibiotic resistance plasmids had been transferred, could not be detected. The reasons for this are unclear, but it may be that genes essential for plasmid transfer are poorly expressed, have become deleted, or have become inserted in the chromosome in some of these strains. Visualization of the plasmids in exconjugants has eliminated the possibility of chromosomal insertion in L. plantarum 1752. Similar observations have been reported when pAMβ1 was transferred to L. casei, and it was

variety of molecular forms, and care is needed in the interpretation of plasmid profiles. All of the plasmids identified in L. plantarum were small, and despite extensive examination by both our rapid lysis procedure and that reported previously (8), we were unable to visualize the three large plasmids reported in L. plantarum 352 (8). Like other lactic acid bacteria examined to date, L. plantarum strains have a large complement of plasmids, which at present are cryptic, but we will now attempt to obtain derivatives of some of these strains which contain single plasmids or are plasmid free, so that the roles of the plasmids can be ascertained.

Frequencies for the conjugative transfer of pAMβ1 and pIP501 to L. plantarum are shown in Table 2. No colonies were observed on control plates. Strains of L. plantarum 1752 to which either pAMβ1 or pIP501 had been transferred were examined for the presence of these plasmids. Use of the large-scale plasmid preparation technique enabled both pIP501 and pAMβ1 to be visualized (Fig. 2). A previous publication (6) has reported that although transfer of pAMβ1-encoded erythromycin resistance to L. casei was

FIG. 1. Plasmid bands visualized in six strains of L. plantarum by the rapid lysis method. Tracks: a, 1752; b, 1193; c, 340; d, 343; e, 704; f, NCDO 352.
suggested that deletion may occur on transfer (6). The position of pAMβ1 and pIP501 from L. plantarum 1752 exconjugants on agarose gels rules out the possibility of a sizeable deletion in this strain at least. We could not transfer these plasmids from L. plantarum 1752 donor strains to S. faecalis JH2-1, but transfer of pAMβ1 to S. lactis 712 was observed. It may be that some host-specific characteristic is able to compensate for the loss of transfer function in the latter case. The host range of these plasmids is limited; in similar experiments we have been unable to transfer either plasmid to two Leuconostoc mesenteroides strains (unpublished observations).

The ability to transfer these plasmids to L. plantarum provides an initial method of genetic transfer and may allow the mobilization of nonconjugative plasmids and other genetic markers. Derivatives of pIP501 are now being extensively developed as cloning vectors for use in gram-positive bacteria, and these should be maintained in L. plantarum, if a suitable means of introduction can be found.

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LITERATURE CITED