Manipulation for Plasmid Elimination by Transforming Synthetic Competitors Diversifies Lactococcus lactis Starters Applicable to Food Products

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This study was designed selectively to eliminate a θ-plasmid from Lactococcus lactis strains by transforming synthetic competitors. A shuttle vector for Escherichia coli and L. lactis, pDB1, was constructed by ligating a partial replicon of pDR1-1B, which is a 7.3 kb θ-plasmid in L. lactis DRC1, with an erythromycin resistance gene into pBluescript II KS⁺. This versatile vector was used to construct competitors to common lactococcal θ-plasmids. pDB1 contains the 5' half of the replication origin and the 3' region of repB of pDR1-1B, but lacks the 1.1-kb region normally found between these two segments. A set of primers, Pv3 and Pv4, was designed to amplify the 1.1-kb middle parts of the general θ-replicons of lactococcal plasmids. When the PCR products were cloned into the Nru I and Xho I sites of pDB1, synthetic replicons were constructed and replication activity was restored. A number of θ-plasmids in L. lactis ssp. lactis and cremoris were eliminated selectively by transforming the synthetic competitors. These competitors were easily eliminated by subculture for a short time in the absence of selection. The resulting variants contained no exogenous DNA and are suitable for food products, since part of the phenotype was altered without altering other plasmids indispensable for fermentation.

Key words: Lactococcus lactis; plasmid incompatibility; plasmid elimination; cheese starter

Strains of lactococcal bacteria are used as starters in the fermentation of dairy products. Such strains generally carry a number of plasmids, varying in size from approximately 2 kb to 80 kb. Some plasmids encode properties essential to the manufacture of dairy products, such as lactose fermentation, proteolysis, diacetyl production, and phage resistance, and others encode nonessential or unknown properties.

Plasmid elimination is a fundamental technique for investigating diverse properties of encoding plasmids. It is currently performed by culturing with a mutagenic chemical such as acridine orange, culturing in unbuffered medium, exposing cells to elevated growth temperatures, regenerating bacterial protoplasts, or a composite of these methods.1–5 With these methods, plasmids cannot be chosen for elimination, and the simultaneous loss of more than one plasmid is frequent. In addition, the resulting variants that have lost co-existing essential plasmids are ineffective as starters.

It is well known that the introduction of a foreign plasmid destabilizes the inheritance of the incompatible resident plasmid. The phenomenon of plasmid instability has been adopted for the classification of incompatibility groups.6,7 It has been reported that many lactococcal plasmids belong to the θ-replicating pCl305-type family, and several plasmids of the θ-family coexist in most wild-type lactococci.8,9 These θ-type plasmids require a replication initiator protein (RepB) and an origin of replication (ori) containing a strongly conserved AT-rich box, a 22-bp iteron sequence tandemly repeated three and a half times, and two sets of inverted repeats.8–11) Sequence comparison of the θ-type replication module showed a high level of nucleotide homology distributed through ori and repB.8,9) Replicon homology is, however, believed to be a main cause of incompatibility, thus excluding coexistence.9) To solve this apparent paradox, detailed studies have been performed on both ori and RepB.8,10,11) Present knowledge of the incompatibility of pCl305-type plasmids indicates that the incompatibility determinant is contained within the 22-bp direct repeat and the first inverted repeat in the ori, and that the specificity of the replication protein to the origin is governed by a residue of several amino acids.9,12)

Previous studies have demonstrated that a growth rate-limiting plasmid, pDR1-1, was eliminated from Lactococcus lactis ssp. lactis DRC1 by the transformation of its own replication module with a selection marker. The maximum specific growth rate (μmax) of the resulting variant was significantly higher than that of the wild-type strain, but the variant retained tolerance to

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antibiotics without selection, so that it was unacceptable in food applications. In this report, we present a manipulation to eliminate an optional lactococcal \( \theta \)-plasmid with plasmid incompatibility by transforming unstable synthetic replicons. A versatile vector, \( pDB1 \), and a set of primers, \( pDB1 \), and a set of primers, \( pV3 \) and \( pV4 \) (used to amplify fragments including the incompatibility determinant encoded by optional \( \theta \)-plasmids in lactococci), were designed to construct the competitors. This procedure is thought to be useful for diversifying fermentation starters for food products.

### Materials and Methods

#### Bacterial strains and plasmids

All the strains and plasmids used in this study are listed in Table 1. \( L.\ lactis \) ssp. \( lactis \) biovar. \( diacetylactis \) DRC1 was obtained from the National Institute for Research in Dairying (now the Agricultural and Food Research Council, AFRC, of the Institute of Food Research, Shinfield, UK), and all other wild-type strains were obtained from our laboratory collection. Tests of the replication proficiency of recombinant plasmids were carried out in \( L.\ lactis \) ssp. \( lactis \) IL1403, previously described as a plasmid-free strain. \( E.\ coli \) XL1-Blue was used for cloning with \( pBluescript \) II (Stratagene Cloning Systems, La Jolla, CA). The plasmid \( p8Em1 \) contained both the erythromycin resistance (Em\(^R\)) gene from \( pAM/J1 \) and the multi-cloning site from \( pUC118 \).

#### Media and cultivation conditions

\( L.\ lactis \) strains were grown in TYG medium (1% tryptone, 0.5% yeast extract, 0.5% sodium chloride, 1% glucose, and 1% sodium succinate, pH 6.8) or sterile skim milk (10% milk solids) at 30 °C. \( pBluescript \) II and recombinant plasmids were maintained in \( E.\ coli \) strain XL1-Blue, which was grown in Luria-Bertani (LB) medium supplemented with ampicillin (Ap) (50 \( \mu \)g/ml) (LB-A) at 37 °C.

#### Plasmid preparation and manipulations

**E. coli** plasmid DNA was isolated using a Plasmid Mini Kit (Qiagen, Chatsworth, CA). Restriction endonucleases (Toyobo, Tokyo), Ligation Kit ver. II (Takara, Ohtsu), a QIAquick Gel Extraction Kit (Qiagen), and a DNA Blunting Kit (Takara) were used as recommended by the respective manufacturers. Lactococcal plasmid DNA was isolated following the method described by Anderson and McKay. Total plasmids from \( L.\ lactis \) ssp. \( lactis \) DRC1, N7, and 527 and \( L.\ lactis \) ssp. \( cremoris \) 712 were extracted from overnight culture in 10 ml of TYG and separated by agarose gel electrophoresis using 0.7% and 1.0% agarose. Individual plasmid bands were stained with ethidium bromide and cut out. Each piece was transferred to a plastic tube and purified using the QIAquick Gel Extraction Kit. Each of the purified plasmids was eluted in 50 \( \mu \)l of distilled water.

#### PCR

PCR was performed using a GeneAmp PCR System 2400 (Perkin-Elmer, Wellesley, MA) and KOD-plus DNA polymerase (Toyobo). Two microliters of 100-fold diluted plasmid solutions were used as PCR templates. The PCR conditions for amplifying fragments containing the 5’ half of the AT-rich box (upstream fragment for construction of synthetic replicons, \( FUb1 \)) and the 3’ region of \( repB \) (downstream fragment for construction of synthetic replicons, \( FDb1 \)) encoded by \( pDR1-1B \) were as follows: denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 53 °C for 30 s, and extension at 68 °C for 45 s, with an additional extension of 7 min at 68 °C after the last cycle. The PCR conditions for amplifying

<table>
<thead>
<tr>
<th>Strains and plasmids</th>
<th>Properties</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strains</strong> ( L.\ lactis ) ssp. ( lactis )</td>
<td>Wild type</td>
<td>32, 33</td>
</tr>
<tr>
<td>DRC1</td>
<td>Wild type</td>
<td>Lab. collection</td>
</tr>
<tr>
<td>N7</td>
<td>Wild type</td>
<td>Lab. collection</td>
</tr>
<tr>
<td>527</td>
<td>Wild type</td>
<td></td>
</tr>
<tr>
<td><strong>Strains</strong> ( L.\ lactis ) ssp. ( cremoris )</td>
<td>Wild type</td>
<td>Lab. collection</td>
</tr>
<tr>
<td>712</td>
<td>Plasmid-free derivative of IL594</td>
<td>14</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( p8Em1 )</td>
<td>( pUC118 ) containing ( pAM/J1 ) Em(^R) gene</td>
<td>16</td>
</tr>
<tr>
<td>( pDR1-1B )</td>
<td>7.3 kb ( \theta )-plasmid from ( L.\ lactis ) DRC1</td>
<td>This study</td>
</tr>
<tr>
<td>( pDB1 )</td>
<td>Receptor vector, a partial replicon of ( pDR1-1B ) with a Em(^R) gene cloned into ( pBluescript ) II, Ap(^R), Em(^R)</td>
<td>This study</td>
</tr>
<tr>
<td>( pCV(x) )</td>
<td>Part of optional ( \theta )-replicon including incompatibility determinant cloned into ( pBluescript ) II, Ap(^R)</td>
<td>This study</td>
</tr>
<tr>
<td>( pBLb1 )</td>
<td>( pDR1-1B ) cloned into the ( Hinc ) II site of ( pBluescript ) II, Ap(^R)</td>
<td>This study</td>
</tr>
</tbody>
</table>

\textbf{Table 1.} \( L.\ lactis \) Strains and Plasmids

Em\(^R\): resistance to erythromycin; Ap\(^R\), resistance to ampicillin

Lab. collection, National Institute of Livestock and Grassland Science collection
the incompatibility determinant encoded by each individual plasmid (VF(X)s) were as follows: denaturation at 94 °C for 2 min followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 45 °C for 30 s, and extension at 68 °C for 2 min, with an additional extension of 7 min at 68 °C after the last cycle.

**DNA sequence analysis.** Plasmid pDR1-1B (7.3 kb), originally present in *L. lactis* DRC1. pDR1-1B, was cloned into the *Hinc* II site of pBluescript II, generating pBLb1, which was deleted unidirectionally using the Deletion Kit for Kilo Sequencing (Takara). Nested deletion clones were sequenced using a *Taq* dye-primer cycle sequencing kit and an Applied Biosystems 373A automated DNA sequencer (Applied Biosystems, Foster City, CA). The nucleotide sequences of open reading frames (ORFs) and their deduced amino acid sequences were subjected to BLAST and FASTA net searches.

**Transformation conditions.** *E. coli* was transformed following the CaCl₂ method described by Sambrook. The transformants were selected on LB agar plates (1.5% agar) supplemented with Ap (50 μg/ml) and/or Em (500 μg/ml). *L. lactis* was transformed by electroporation with a 2-mm gap electroporation cuvette, as described by Holø and Nes, using a *Bio-Rad* gene pulser (Bio-Rad Laboratories, Richmond, CA). For electroporation, 40 μL of competent cells was mixed with 10 ng of plasmid DNA introduced into an ice-cooled electroporation cuvette, and an electrical pulse (25 μF, 200 Ω, and 2.5 kV) was delivered. The transformants containing recombinant plasmids were selected on SR agar plates (1% tryptone, 0.5% yeast extract, 1% glucose, 20% sucrose, 2.5% gelatin, 2.5 mM MgCl₂, 2.5 mM CaCl₂, and 1.5% agar, pH 6.8) with Em (5 μg/ml).

**Measurement of plasmid stability.** Cultures of transformants containing recombinant plasmids were inoculated into fresh TYG-E medium and grown for 12 h (T = 0), after which they were diluted (0.1%) in fresh TYG and grown to the stationary phase (approximately 10 generations). Ten identical transfers in TYG medium were carried out to reach 100 generations (T = 100). At T = 0, T = 50, and T = 100, dilutions of the cultures were plated onto TYG agar plates and incubated at 30 °C for 24 h, after which 100 colonies were replica-plated onto TYG and TYG-E agar plates and incubated at 30 °C for 24 h. Percentages of the colonies of the transformants containing recombinant plasmids in the population were calculated for T = 0, T = 50, and T = 100.

**Measurement of acidifying activity.** The acidifying activity was evaluated by measuring the pH reached in sterile 10% (w/v) reconstituted skim milk (Snow Brand Milk Products, Tokyo) from a 1% inoculum after 6, 12, and 24 h of growth at 30 °C.

**Measurement of citrate utilization.** The ability of citrate uptake to produce diacetyl and acetoin was determined spectrophotometrically by the method of Fryer.

**Nucleotide sequence accession number.** The plasmid pDR1-1B sequence has been assigned DDBJ accession no. AB079380.

**Results and Discussion**

Selection of a plasmid for vector construction

*L. lactis* DRC1 carried more than five plasmids, including two multicopy-plasmids, pDR1-1 and pDR1-1B. The former has been found to affect the μₘₐₓ of host cells significantly. In contrast, the latter had no effect on the μₘₐₓ of host cells, but they were indistinguishable by agarose gel electrophoresis (7,412 bp and 7,344 bp respectively), and they had almost same the restriction sites. The entire nucleotide sequence of the two plasmids revealed that each plasmid contained a typical replication module of plasmids belonging to the lactococcal θ-family. The θ-replication modules of lactococcal plasmids are often associated with two additional coding regions, encoding a replication-associated protein (OrfX) and a specificity subunit of a type I restriction-modification system (HsdS), which appear to be part of the same transcriptional unit as *repB*.

**Construction of a versatile vector pDB1 to enable synthetic replicons to replicate in *L. lactis***

The replication module of pDR1-1B was 100% identical to that of the 8.7-kb plasmid pCI305 from *L. lactis* ssp. *lactis* UC317, the 6.2-kb plasmid pCI3 from *L. lactis* ssp. *cremoris* UC509.9, and the 6.2-kb plasmid pAH33 from *L. lactis* ssp. *lactis* DPC721. Based on its relatively high copy number per cell and wide distribution among *L. lactis* ssp. *lactis* and *cremoris*, the replication module of pDR1-1B was chosen to serve as the starting material for the construction of a versatile vector.
Maps of pDR1-1 and pDR1-1B (A) and the Replication Module of pDR1-1B (B).

A, Maps of pDR1-1 and pDR1-1B. Relevant features of the plasmids and noticeable restriction sites are indicated. Bg, Fs, Ec, Pv, Sa, Kp, Sp, Hc, Xb, and Bm indicate the BglII, FspI, EcoRI, PvuII, SacI, KpnI, SphI, HinClI, and XbaI sites respectively. The PCR primers Pc1, Pc2, Pv3, Pv4, Pc5, and Pc6 are illustrated at their positions. Thin arrows indicate ORFs. Closed squares and open squares indicate AT-rich boxes and 22-bp direct repeats respectively. Identical regions between two plasmids are represented by thick lines. The replication module of pDR1-1B is represented by a dashed line and is illustrated in Fig. 1B. B, Replication module of pDR1-1B. The \( \text{RBS RepB} \) box of repB promoter and RBSs are indicated in boldface. AT-rich boxes among the \( \text{replicons} \) are boxed. The solid arrows indicate 22-bp direct repeats. The dashed arrows indicate two inverted repeats, IR1 and IR2. The one-letter code was used for deduced amino acid sequences. The thin line indicates the conserved domain of RepB for protein dimerization. The double line indicates the conserved domain for copy number control. The thick line indicates the conserved domain for governing ori-specific interactions. The dashed line indicates conserved amino acid sequences, which were located from 249 to 272, among lactococcal \( \text{replicons} \).

The PCR primers Pc1 (5' - AACGCTCTAAAAATCGATTTAAGCGA - 3'), Pc2, Pv3, Pv4, Pc5 and Pc6 (5' - CTGGAGAGTATCATCTGCTTCATCAATA - 3') are illustrated at their positions, and nucleotide substitutions are indicated in boldface.
designed to amplify the fragment of the upstream part (FUb1) and the downstream part (FDb1) of the replication module. Pc2 corresponded to sequences in the AT-rich box of ori and FUb1 contained the 5′ half of this region. FDb1 contained the 3′ region of repB, encoding a C-terminal part of RepB (position, 276 to 386) (Fig. 1). FUb1 (a 445-bp fragment), FDb1 (a 466-bp fragment), and the EmR gene were digested with restriction enzymes and cloned one after another into the BamHI-EcoRI site, the PstI-XbaI site, and the SacI site respectively of pBluescript II KS+, generating a vector pDB1 (Fig. 2A). pDB1 was lacking the 1.1-kb middle part of the replicon, containing the incompatibility determinant of pDR1-1B and the ability to replicate in L. lactis.

**Construction of synthetic replicons able to restore replication activity**

A 1.1-kb PCR fragment containing the incompatibility determinant of pDR1-1B, designated VF5, was amplified from pBLb1 as a template using Pv3 and Pv4. VF5 contained 22-bp direct repeats, two inverted repeats, and a 5′-region of repB, encoding an N-terminal to the middle part of RepB (position, 1 to 275) (Fig. 1). Within this truncated RepB, regions presumably implicated in dimerization (a leucine zipper motif, position, 31 to 45), copy number control (position, 129 to 144), and DNA binding (a helix-turn-helix motif, position, 215 to 241) were identified based on their similarity to the consensus sequence of RepB proteins. It has been suggested that this last motif contains a 13-amino acid stretch (position, 216 to 228) of the region governing ori-specific interactions for lactococcal plasmids. After XhoI digestion, the VF5 was cloned into the NruI and XhoI sites of pDB1, generating a 6.1-kb synthetic replicon pCV5. When pCV5 was transformed into L. lactis IL1403, all the EmR transformants carried Pc6 are strongly conserved among repB encoded by lactococcal plasmids.

**Design of PCR primers to amplify the incompatibility determinant of pDR1-1B**

A set of primers, Pv3 and Pv4 (containing three substitutions generating an XhoI site), was designed to amplify the middle part of the pDR1-1B replication module (Fig. 1). The sequences of Pv3 and Pv4 corresponded to sequences at the AT-rich box and the middle region of repB respectively. It has been found that the overlapping nucleotide sequences of Pv4 and Pc6 are strongly conserved among repB encoded by lactococcal plasmids.

**Construction of synthetic replicons able to restore replication activity**

A 1.1-kb PCR fragment containing the incompatibility determinant of pDR1-1B, designated VF5, was amplified from pBLb1 as a template using Pv3 and Pv4. VF5 contained 22-bp direct repeats, two inverted repeats, and a 5′-region of repB, encoding an N-terminal to the middle part of RepB (position, 1 to 275) (Fig. 1). Within this truncated RepB, regions presumably implicated in dimerization (a leucine zipper motif, position, 31 to 45), copy number control (position, 129 to 144), and DNA binding (a helix-turn-helix motif, position, 215 to 241) were identified based on their similarity to the consensus sequence of RepB proteins. It has been suggested that this last motif contains a 13-amino acid stretch (position, 216 to 228) of the region governing ori-specific interactions for lactococcal plasmids.

**After XhoI digestion, the VF5 was cloned into the NruI and XhoI sites of pDB1, generating a 6.1-kb synthetic replicon pCV5. When pCV5 was transformed into L. lactis IL1403, all the EmR transformants carried**
pCV5, indicating that pCV5 restored replication activity. pCV5 included substitutions of one nucleotide in the AT-rich box and three nucleotides in the repB consensus sequences at the junction of pDB1 and VF5 (no translational alteration of amino acid sequences resulted from substitutions in repB) (Fig. 1). This suggests that the modifications to the four nucleotides were not fatal to autonomous replication of the synthetic pCV5.

**Table 1**

<table>
<thead>
<tr>
<th>Host Strain</th>
<th>Plasmid</th>
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<tr>
<td>DRC1</td>
<td>pDR1-1B</td>
</tr>
<tr>
<td></td>
<td>pCV5</td>
</tr>
<tr>
<td>527</td>
<td>pCVL3</td>
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<tr>
<td>712</td>
<td>pCVL1</td>
</tr>
<tr>
<td>N7</td>
<td>pCVc8</td>
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<td>pCVL1</td>
</tr>
<tr>
<td>N7</td>
<td>pCVc8</td>
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</tbody>
</table>

Fig. 3. Alignment of the Replication Origins of pDR1-1B and Synthetic Hybrid Replicons. Sequences identical in all are indicated by asterisks. AT-rich boxes are boxed.

Application of optional θ-plasmid elimination from *L. lactis* ssp. lactis and cremoris using incompatible synthetic replicons

Total plasmid DNA was prepared from DRC1, N7, 527, and 712. Each individual plasmid was isolated by electrophoresis and purified to serve as a PCR template. PCR products were cloned into pDB1, generating synthetic hybrid replicons pCV(X)s. The construction of pCV(X)s and the manipulation for plasmid elimination from *L. lactis* ssp. lactis and cremoris using incompatible synthetic replicons.
tion with pCV(X)s are outlined in Fig. 2A and B. Eight synthetic replicons (pCV1, pCV5, and pCV28 from DRC1; pCVc8 from N7; pCVL3 from 527; and pCVL1, pCVL10, and pCVm6 from 712) were obtained from four strains, including L. lactis ssp. cremoris and L. lactis (Fig. 3). All synthetic replicons replicated in L. lactis IL1403. This indicates that various synthetic replicons are constructed from optional lactococcal θ-plasmids, varying in size to approximately 50 kb, in addition to pDR1-1B, using this procedure.

The pCV(X)s were transformed into wild-type L. lactis strains (DRC1, N7, 527, and 712) to eliminate the incompatible plasmids. pCV5, pCV1, pCVc8, and pCVm6 encoded incompatibility determinants of pDR1-1B and pDR1-1 in DRC1, an 8.3-kb plasmid in N7, and a 9-kb plasmid in 712 respectively. These four competitors were successfully introduced in the native hosts. After subculturing up to 100 generations in TYG-E media, the resident plasmids incompatible with each competitor were eliminated selectively. The original replicons of pCV5 and pCV28 were compatible in L. lactis DRC1, but pCV5 and pCV28 differed in only three nucleotides in each of the ori sequences (Fig. 3). These results indicate that the competitors discriminated between compatible and incompatible plasmids with a difference of only three nucleotides.

The 8.3-kb plasmid, which was the source of pCVc8, was expected to be the citrate permease plasmid (CitP-plasmid), because VFc8 of pVCc8 showed the highest level of similarity to the replication region of the CitP-plasmid, and the 8.3-kb plasmid-eliminated variant of L. lactis N7 lost the ability to metabolize citrate and to produce the aromatic compound diacetyl from citrate. The sequences of pCVc8 and pCVL10 were in at least 99% agreement (Fig. 3). However, the source of pCVL10 was large (> 40 kb), and was from L. lactis ssp. cremoris 712. The 712 strain is unable to metabolize citrate, and no example has yet been reported of a citrate plasmid from L. lactis ssp. cremoris. These results suggest that the replication module of the citrate plasmid in lactis strains should be assigned to a plasmid larger than the citrate plasmid in cremoris strains.

No transformants were obtained by transformation of pCVL1 and pCVL10 into 712, pCV28 into DRC1, or pCVL3 into 527. All the incompatible plasmids of these four pCV(X)s were similar in that they were large (e.g., the Lac-Prt plasmid).

### Table 2. Transformation Efficiency and Stability of pCV(X) in IL1403

<table>
<thead>
<tr>
<th>Tested plasmid</th>
<th>Transformation efficiency*</th>
<th>Em&lt;sup&gt;a&lt;/sup&gt; colony at T = 0**</th>
<th>Em&lt;sup&gt;a&lt;/sup&gt; colony at T = 50**</th>
<th>Em&lt;sup&gt;a&lt;/sup&gt; colony at T = 100**</th>
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<tr>
<td>pCV1</td>
<td>8.1 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>pCV5</td>
<td>9.8 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>pCV28</td>
<td>7.8 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pCVc8</td>
<td>1.2 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pCVL3</td>
<td>5.0 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>pCVL1</td>
<td>3.2 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>pCVL10</td>
<td>6.2 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>pCVm6</td>
<td>8.3 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>100</td>
<td>24</td>
<td>0</td>
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</tbody>
</table>

Em<sup>a</sup>, resistance to erythromycin
*Number of transformants per microgram of pCV(X) DNA
**Percentage of Em<sup>a</sup> colonies in the population

Concluding Remarks

Using the present technique, middle-sized (approximately 10 kb) multiple plasmids were eliminated quickly and efficiently. Through comparisons between plasmid variants and the wild-type strain, the properties encoded by the curing plasmid can be inferred. Moreover, the phenotypic characterizations regulated by both the eliminating plasmid and other co-existing plasmids or chromosome can be revealed. Hence, we are positive that this method is a useful strategy for investigating various plasmids in L. lactis. In particular, it is worth testing this method for the elimination of stable plasmids resistant to current methods.

After manipulation for plasmid elimination, competitors containing synthetic replicons disappeared on short-term cultivation without selection. The resulting variants contained no exogenous DNA, and were suitable for food products because part of the phenotype could be changed without altering other plasmids indispensable for fermentation. Hence, this procedure...
is thought to be useful not only for investigating the properties of individual plasmids, but also for diversifying fermentation starters for food products.

References


