Novel sucrose transposons from plant strains of *Lactococcus lactis*

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Abstract

*Lactococcus lactis* strains isolated from vegetable products transferred the ability to ferment sucrose in conjugation experiments with the recipient strain *L. lactis* MG1614. Nisin production and sucrose fermentation were transferred together from two strains, but transfer also occurred from several other strains which did not produce nisin. Pulsed-field gel electrophoresis analysis showed that all transconjugants had acquired large chromosomal insertions at two main sites. Nisin–sucrose transconjugants had gained inserts of 70 kb, while those that fermented sucrose without nisin production contained inserts of between 50 and 110 kb. Transconjugants from one donor had acquired a separate insertion of 55 kb which correlated with enhanced bacteriophage resistance, but contained neither nisin nor sucrose fermentation genes. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Conjugative transposon; Sucrose metabolism; Nisin; Bacteriophage resistance; *Lactococcus lactis*

1. Introduction

It is well established that biosynthesis of the bacteriocin nisin and the ability to ferment sucrose are genetically linked as part of a 70-kb conjugative transposon in *Lactococcus lactis* subsp. *lactis* [1–4]. This transposon is able to integrate itself into the lactococcal chromosome with one preferred site of insertion in strain MG1614 [3]. Resistance to small isometric bacteriophage [1,5], production of N5-(carboxyethyl)ornithine synthase [6], and altered peptidoglycan composition [7] have also been associated with the nisin–sucrose element. A non-lactococcal origin of this transposon is supported by G+C percent and codon usage data [8,9], and differences in phenotype, conjugative ability, nisin type and transposon architecture suggest that a heterogeneous group of nisin–sucrose transposons exists [9].

We have recently described nisin producing strains of *L. lactis* isolated from fruit and vegetable products [10], and several other strains which fermented sucrose but did not produce nisin were isolated from the same environment. This paper describes novel conjugative elements from these strains in which the genes for sucrose metabolism are not linked with those for nisin production.

2. Materials and methods

2.1. Bacterial strains and media

The lactococci used in this study are shown in Table 1, and were grown at 28°C in M17 broth supplemented with 0.5% (w/v) glucose, or 0.5% sucrose. Indicator agars [11] were used to differentiate lactococci.

2.2. Conjugation

Conjugal matings were set up between sucrose fermenting strains and the plasmid-free recipient strain MG1614 [12] as previously described [10]. Selection was on SIA supplemented with 600 μg ml⁻¹ streptomycin.

2.3. Pulsed-field gel electrophoresis (PFGE)

Suc⁺ transconjugants and the MG1614 recipient strain were prepared for PFGE analysis as described previously [10]. DNA was digested for 16 h with Smal, or I-CeuI (New England Biolabs, Beverly, MA, USA). Gels were run using either a CHEF DR II pulsed-field gel apparatus...
(Bio-Rad Laboratories, Richmond, CA, USA), or with a FIGE Mapper electrophoresis system (Bio-Rad). DNA fragments are referred to by the numbering used on the physical and genetic map of strain MG1363 [13], the parent of strain MG1614.

### 2.4. DNA methodology

Pulsed-field gels were hybridized with probes for the nisin structural gene, and the sucrose hydrolase gene constructed by amplification of lactococcal DNA with primers already described [14,15]. Sequence data on the nisin gene cluster [16] were used to prepare primers to the nisT gene.

### 2.5. Bacteriophage sensitivity

Changes in bacteriophage sensitivity resulting from the inserted DNA were determined by measuring the efficiency of plating of small isometric (Psk1, P712, P742, P1738 and P1760) and prolate (Pc2) lactococcal bacteriophage on the recipient and transconjugant strains.

### 3. Results

#### 3.1. Transfer of sucrose metabolism

The sucrose fermenting phenotype was transferred to MG1614 from 9 of 38 Suc⁺ donor strains at frequencies between $10^{-4}$ and $10^{-7}$ CFU per donor cell (Table 1). Transfer occurred from 2 of 22 nisin producing strains and 7 of 16 non-producers. Acquisition of sucrose fermentation could not be correlated with any change in plasmid pattern in the transconjugants (data not shown).

#### 3.2. PFGE and sites of DNA insertion

The PFGE patterns for SmaI digests of genomic DNA from the donor and recipient strains have been reported previously [10]. The Suc⁺ transconjugants showed PFGE patterns closely similar to the MG1614 recipient strain, but with inserts of different sizes and at different sites (Fig. 1A and Table 2). Correlation between the inserted DNA and the phenotypic changes that occurred was established using probes to the nisA, nisT and sucrose hydrolase genes (Fig. 1B,C).

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Table 1

<table>
<thead>
<tr>
<th>Lactococcus strains used and frequencies of sucrose metabolism transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>MG1614</td>
</tr>
<tr>
<td>KF5⁺</td>
</tr>
<tr>
<td>KF31⁺</td>
</tr>
<tr>
<td>KF152⁺</td>
</tr>
<tr>
<td>KF165</td>
</tr>
<tr>
<td>KF169</td>
</tr>
<tr>
<td>KF201</td>
</tr>
<tr>
<td>KF225</td>
</tr>
<tr>
<td>KF241⁺</td>
</tr>
<tr>
<td>KF292</td>
</tr>
</tbody>
</table>

*RaF, raflnose; Lac, lactose; Suc, sucrose; Nis, nisin production; Bac, bacteriocin production, Sm, streptomycin.

*These strains are closely related but are distinguishable by slight differences in PFGE and plasmid profiles [10].
3.3. Properties of transconjugants

Suc⁺ transconjugants were unable to ferment lactose or raffinose, but did show differences in nisin production and phage resistance when compared to the MG1614 recipient strain (Table 2). Strains KF1113 and KF1116 show insertion of a 70-kb nisin–sucrose element into the MG1614 chromosome at the same sites that have previously been described with nisin-producing transconjugants [1–3]. Most transconjugants from the KF201 donor show insertion at the same sites as the nisin–sucrose element but differ in that the inserted fragment is approximately 15 kb smaller and does not contain the nisin genes. In KF1119 insertion was into Smal fragment Sm3 which is not recorded as a site for the nisin–sucrose element, but sucrose fermentation was not stable in this strain. The 110-kb insert in KF1148 is also unstable and is probably the result of insertion of two copies of the transposon.

The donor strains KF31 and KF152 are closely related [10], and their transconjugants also show inserts (of approximately 50 kb) into the same Smal fragments as the nisin–sucrose elements. However, with these donors insertion into fragment Sm2 was unstable and the Suc⁺ phenotype was rapidly lost during subculturing. The Smal digest DNA from KF1112 and its sucrose negative derivative (KF1112-1) also showed a new band at 52 kb which did not hybridize with the nisin or the sucrose probes. These strains showed an insertion into the 38-kb fragment Sm12, and there was also additional DNA at 42 kb (Fig. 2A). Digestion with I-CeuI showed that this insertion increased the 80-kb Ce4 fragment to approximately 135 kb, whereas the main site for insertion of sucrose or nisin–sucrose elements is into the Ce2 fragment (Fig. 2B). Strains containing this extra fragment showed greater phage resistance, including resistance to prolate phage, than did the other transconjugants. The other strains related to KF31 (Table 1) all gave transconjugants which harbored this additional 55-kb fragment (data not shown).

4. Discussion

This is the first report of conjugative transposon-like elements that encode sucrose metabolism without nisin production in L. lactis, and follows the report of a large conjugative sucrose transposon in enterobacteria [17]. The results suggest that a family of related sucrose transposons exists in Lactococcus, and that those described here represent a new sub-class of these elements. It is likely that the

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Table 2

Properties of transconjugants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Donor</th>
<th>Insert size</th>
<th>Insert site</th>
<th>Phage resistancea</th>
</tr>
</thead>
<tbody>
<tr>
<td>KF1113</td>
<td>KF165</td>
<td>70 kb</td>
<td>Sm2/Sm5B</td>
<td>+</td>
</tr>
<tr>
<td>KF1116</td>
<td>KF165</td>
<td>70 kb</td>
<td>Sm5B</td>
<td>+</td>
</tr>
<tr>
<td>KF1119</td>
<td>KF201</td>
<td>55 kb</td>
<td>Sm3</td>
<td>(+)b</td>
</tr>
<tr>
<td>KF1141</td>
<td>KF201</td>
<td>55 kb</td>
<td>Sm5B</td>
<td>+</td>
</tr>
<tr>
<td>KF1142</td>
<td>KF201</td>
<td>55 kb</td>
<td>Sm2</td>
<td>+</td>
</tr>
<tr>
<td>KF1144</td>
<td>KF201</td>
<td>55 kb</td>
<td>Sm2/Sm5B</td>
<td>+</td>
</tr>
<tr>
<td>KF1148</td>
<td>KF201</td>
<td>110 kb</td>
<td>Sm5B</td>
<td>(+)b</td>
</tr>
<tr>
<td>KF1131</td>
<td>KF152</td>
<td>50 kb</td>
<td>Sm2</td>
<td>+</td>
</tr>
<tr>
<td>KF1111</td>
<td>KF31</td>
<td>50 kb</td>
<td>Sm2</td>
<td>(+)b</td>
</tr>
<tr>
<td>KF1112</td>
<td>KF31</td>
<td>50 and 55 kb</td>
<td>Sm2 and Sm12</td>
<td>(+)b</td>
</tr>
<tr>
<td>KF1112-1</td>
<td>KF31</td>
<td>55 kb</td>
<td>Sm12</td>
<td>+</td>
</tr>
</tbody>
</table>

aSI, small isometric phage; P, prolate phage.
bThe inserted DNA and the sucrose phenotype are unstable in these strains.
nisin–sucrose elements have arisen by the integration of nisin genes into a genetic element encoding sucrose, conjugal transfer and transposition genes as has previously been proposed [2]. The conjugal element from these plant strains show the same site specificity as the documented nisin–sucrose transposons, but are smaller and lack the nisin genes. The 15–20 kb difference in size between the transposons with and without the nisin genes is in agreement with the size quoted for the complete nisin gene cluster [18].

The results highlight the untapped genomic diversity of *L. lactis* as many of the donor strains were also able to transfer a second conjugal element encoding raffinose metabolism that inserted at different sites in the MG1614 chromosome [15]. Three different chromosomal segments each of ~50 kb can be transferred to specific sites on the MG1614 chromosome during matings with the strains similar to KF31. One of these insertions, the 55-kb fragment in KF1112-1 could be correlated with enhanced phage resistance. Use of these transferable elements to modify the properties of industrial lactococcal strains is being investigated.

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**References**


