Distribution and Evolution of Nisin-Sucrose Elements in Lactococcus lactis

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The distribution, architecture, and conjugal capacity of nisin-sucrose elements in wild-type Lactococcus lactis strains were studied. Element architecture was analyzed with the aid of hybridizations to different probes derived from the nisin-sucrose transposon Tn5276 of L. lactis NIZO R5, including its left and right ends, the nisA gene, and IS1068 (previously designated iso-IS904), located between the left end and the nisA gene. Three classes of nisin-sucrose elements could be distinguished in the 13 strains investigated. Classes I and II consist of conjugative transposons containing a nisA gene and a nisZ gene, respectively. Representative conjugative transposons of these classes include Tn5276 (class I) from L. lactis NIZO R5 and Tn5278 (class II) from L. lactis ILC11. The class II transposon found in L. lactis NCK400 and probably all class II elements are devoid of IS1068-like elements, which eliminates the involvement of an iso-IS1068 element in conjugative transposition. Members of class III contain a nisZ gene, are nonconjugal, and do not contain sequences similar to the left end of Tn5276 at the appropriate position. The class III element from L. lactis NIZO 22186 was found to contain an iso-IS1068 element, termed IS1069, at a position corresponding to that of IS1068 in Tn5276 but in the inverted orientation. The results suggest that an iso-IS1068-mediated rearrangement is responsible for the dislocation of the transposon’s left end in this strain. A model for the evolution of nisin-sucrose elements is proposed, and the practical implications for transferring nisin A or nisin Z production and immunity are discussed.

Interest in the structure, function, and application of the lantibiotic nisin, produced by a number of Lactococcus lactis strains, has led to the identification of conjugative transposons carrying the genetic information for nisin biosynthesis, sucrose fermentation, and other traits (for a review, see reference 29). The recently described transposons Tn5276 from strain NIZO R5 (27), Tn5301 from strain NCFB 894 (15), and Tn5307 from strain ATCC 11454 (2, 11, 33) are all approximately 70 kb in size and show a similar organization. Upstream of the nisin structural gene, nisA, the transposons contain an identically orientated insertion sequence (IS) element, designated IS904 in Tn5301 (4) and IS1068 (previously designated IS904 or iso-IS904) in Tn5276 (26, 27). The nucleotide sequence of IS1068 differs from that of IS904 in eight positions, and as a consequence, it has an increased coding capacity (25). The nucleotide sequences of the ends of Tn5276 and Tn5301 are identical, and both contain a single copy of the IS element that is separated from the left end by approximately 250 bp (15, 27). In contrast to earlier suggestions (2, 4, 33), it is unlikely that this IS element is involved in conjugative transposition, since (i) the sites at which Tn5276 and Tn5301 were found to insert show no homology to the IS element and (ii) excision of the ends of Tn5276 was found to depend on the activity of the int and xis genes, which are located at the right end of Tn5276 (28).

Recently, it was found that there are two natural variants of nisin, nisin A and nisin Z, encoded by the nisA and nisZ genes, respectively (3, 22). Both variants contain dehydrated amino acids and lanthionine rings (13, 22), but they differ in a single amino acid (Asn instead of His in nisin Z at position 27).

Because of this substitution, nisin Z shows larger inhibition zones in agar diffusion assays than nisin A (3). It was found that the nisA and nisZ genes are distributed equally among 23 naturally occurring, sucrose-fermenting L. lactis strains (3). All conjugative transposons studied to date code for the production of nisin A. Since conjugal transfer of the ability to produce nisin is of considerable significance in industrial strain improvements (1, 8, 12), it was of interest to determine whether the production of nisin Z could also be conjugally transferred simultaneously with the capacity to ferment sucrose. Therefore, we compared the conjugal capacity of nisin Z- and nisin A-producing strains and examined the organization of their nisin-sucrose elements. We found that the L. lactis nisin-sucrose elements are heterogeneous and can be grouped into three classes based on their architecture. A new iso-IS1068 element, designated IS1069, was characterized in L. lactis NIZO 22186, which produces nisin Z (22). There appeared to be a strong correlation between the integrity of the extreme ends of the investigated elements and the ability to conjugally transfer sucrose fermentation and the production of nisin.

MATERIALS AND METHODS

Bacterial strains and culture and conjugation conditions. Escherichia coli TG1 was used as a host for M13-derived vectors (30). The L. lactis strains used are listed in Table 1. Conjugal matings and the identification of transconjugants were carried out as described previously (27).

DNA manipulations and hybridizations. Bacteriophage and plasmid DNAs were isolated from E. coli essentially by established protocols (30). Isolation of total L. lactis DNA was carried out as described previously (27). DNA was digested with restriction enzymes (Gibco/BRL Life Technologies, Gaithersburg, Md.) as recommended by the manufacturer and separated by agarose gel electrophoresis as described before.
DNA sequencing. The nucleotide sequences of both strands of the iso-IS1068 element (IS1069) upstream of the nisZ gene of *L. lactis* NIZO 22186 and its surrounding regions were determined by the dyeoxy chain termination method (31) adapted for Sequenase version 2.0 (U.S. Biochemical Corp.) with overlapping restriction fragments cloned into M13mp18 and M13mp19 (36) and by using either the M13 universal primer or synthesized primers. Oligonucleotides were synthesized on a Cyclone DNA synthesizer (MilliGen Biosearch Division, San Rafael, Calif.). The sequence data were assembled and analyzed with the PC/Genie program, version 5.01 (Genofit, Geneva, Switzerland).

PCR. PCR experiments were performed in reaction mixtures composed as described previously (19) containing approximately 100 ng of *L. lactis* total DNA as a template. The oligonucleotide primers used were nis10 (5′-GGATAGTATCTCATGTCTGAAC) and nis5 (5′-CCAAAGCAAAACTATACAGC) (Table 1), located in the nucleotide sequence of the nisZ gene of *L. lactis* NIZO 22186 (22) in combination with either IS-I or IS-II. PCR was performed in 30 cycles, each cycle consisting of a denaturing step at 93°C for 1 min, a primer-anneling step at 45°C for 1.5 min, and an extension step at 72°C for 2.5 min, in a Biomed Thermocycler 60 (ICN Biomedicals, Inc., Amsterdam, The Netherlands).

Nisin bioassays and differentiation. Supernatants of *L. lactis* strains were analyzed for the production of nisin by the agar diffusion assay with *Micrococcus luteus* as an indicator (3). Differentiation between the production of nisin A and nisin Z was obtained by analyzing supernatant samples concentrated by hydrophobic interaction chromatography by reversed-phase high-pressure liquid chromatography (HPLC) as described before (3, 22).

Nucleotide sequence accession number. The sequence described here has been assigned GenBank accession number X78469.

### RESULTS AND DISCUSSION

Conjugative transfer of sucrose fermentation and the production of nisin Z and nisin A. Nisin Z was initially found to be produced by *L. lactis* NIZO 22186, which also ferments sucrose (22). Numerous attempts to conjugally transfer the capacity to ferment sucrose and nisin Z production from strain NIZO 22186 to *L. lactis* MG1614 or other lactococcal strains failed (data not shown). In contrast, transfer of Tn5276, specifying nisin A production, from *L. lactis* NIZO R5 was achieved easily under the same conditions (27). Therefore, other available nisin Z producers were screened for their capacity to conjugally transfer the ability to ferment sucrose to strain MG1614 (Table 1). Three of seven *L. lactis* strains containing the nisZ gene were found to transfer the capacity to ferment sucrose and nisin production, with frequencies similar to those for transfer of Tn5276 from strain NIZO R5 (10<sup>-8</sup> to 10<sup>-6</sup> CFU per CFU of donor; transfer frequencies expressed per CFU of recipient are approximately 10-fold lower because of the nisin produced by the donor strains). A transconjugant obtained from the mating of strains ILC11 and MG1614 was studied in more detail, and, as expected, produced nisin Z (data not shown). This and other studies (see below) indicated that nisin Z production was also encoded by a conjugative transposon which also carried the genes for sucrose utilization. The transposon present in *L. lactis* ILC11 was designated Tn5278 (registered with the Plasmid Reference Centre [20]).

To determine whether conjugally inactive nisin-sucrose elements are found only among *L. lactis* strains carrying the nisZ gene, five nisin A producers other than strain NIZO R5, which

### TABLE 1. *L. lactis* strains used in this study and classification of their nisin-sucrose elements

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source&lt;sup&gt;a&lt;/sup&gt; (reference)</th>
<th>Type of nisin gene&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Conjugative capacity&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Class&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
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<tr>
<td>NIZO R5</td>
<td>NIZO (6)</td>
<td>nisA</td>
<td>+</td>
<td>I</td>
</tr>
<tr>
<td>INRA 2</td>
<td>INRA (3)</td>
<td>nisA</td>
<td>+</td>
<td>I</td>
</tr>
<tr>
<td>INRA 3</td>
<td>INRA (3)</td>
<td>nisA</td>
<td>+</td>
<td>I</td>
</tr>
<tr>
<td>INRA 5</td>
<td>INRA (3)</td>
<td>nisA</td>
<td>+</td>
<td>I</td>
</tr>
<tr>
<td>N21</td>
<td>UP (3)</td>
<td>nisA</td>
<td>+</td>
<td>I</td>
</tr>
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<td>ILC (3)</td>
<td>nisA</td>
<td>+</td>
<td>I</td>
</tr>
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<td>ILC11</td>
<td>ILC (3)</td>
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<td></td>
</tr>
<tr>
<td>ILCpSL5</td>
<td>ILC (3)</td>
<td>nisZ</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>NCK400</td>
<td>NCSU (14)</td>
<td>nisZ</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>NIZO 22186</td>
<td>NIZO (22)</td>
<td>nisZ</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>ILC19</td>
<td>ILC (3)</td>
<td>nisZ</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>ILC126</td>
<td>ILC (3)</td>
<td>nisZ</td>
<td>III</td>
<td></td>
</tr>
<tr>
<td>ILCpSL20</td>
<td>ILC (3)</td>
<td>nisZ</td>
<td>III</td>
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</tr>
<tr>
<td>MG1614</td>
<td></td>
<td></td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>


<sup>b</sup> From reference 3.

<sup>c</sup> +, conjugal; -, nonconjugal (results of 3 to 10 independent conjugation experiments).

<sup>d</sup> For definitions of classes, see the text.

Plasmid-free, streptomycin- and rifampin-resistant strain was used as the recipient in conjugation experiments.

(30). DNA fragments were recovered from agarose gels with a USBioian kit (U.S. Biochemical Corp., Cleveland, Ohio) or transferred to GeneScreen Plus nylon membranes (Du Pont NEN Research Products, Wilmington, Del.) with the use of a VacuGene XL unit (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) and the alkaline blotting protocol supplied by the manufacturer. Hybridization, washing, and deprobing conditions were as recommended by the membrane manufacturer.

As a probe for sequences homologous to the left end of Tn5276 (Tn5276-L probe), an oligonucleotide with the sequence 5′-CCAAAGCAAAACTACAGC (nucleotides 107 to 126 in the sequence of the Tn5276 left end [27]) was used at a hybridization temperature of 54°C. As probes for IS1068-like sequences, oligonucleotides with the sequences 5′-GAGCTGAGGGCTACTCA (IS-I probe; corresponding to positions 1090 to 1104 of the transposase gene sequence [EMBL accession number X52273]) (26) and 5′-GGATAGTATCTCATGTCTGAAC (IS-II probe; complementary to positions 150 to 166 of the transposase gene sequence) were used at a hybridization temperature of 48°C. As a probe for the nisA and nisZ genes, an oligonucleotide with the sequence 5′-ATGGGGTTGTAATATGAAAAAC (nis probe [27]) was used at a hybridization temperature of 45°C. Two different probes homologous to sequences at the right end of Tn5276 were used. One was an oligonucleotide with the sequence 5′-GGTATGCTCTCTTTGTTAG (Tn5276-R probe; complementary to nucleotides 118 to 135 in the nucleotide sequence of the Tn5276 right end [27]) that was used at a hybridization temperature of 50°C. The other was the 2.3-kb HindIII–EcoRI fragment from pNZ774 (27), located at the right end of Tn5276, that was hybridized at 65°C. The last probe was labeled by nick translation with [α-<sup>32</sup>P]ATP, whereas all other oligonucleotides were end labeled with [γ-<sup>32</sup>P]ATP as described before (30).
carries Tn5276, were also tested for the capacity to transfer sucrose proficiency and nisin production (Table 1). Except for strain INRA 3, all nisin A producers contained nisin-sucrose elements that could be readily transferred in conjugal matings.

A likely explanation for these results is that nisin-sucrose elements similar to Tn5276 and Tn5278 were present in all strains but that in some cases the transposon had lost its conjugative capacity.

**Heterogeneity of nisin-sucrose elements in L. lactis.** To characterize the Tn5276- and Tn5278-like elements in the 13 strains studied at the DNA level, we hybridized HindIII digests of total DNAs to a probe for the nisA and nisZ genes (nis probe). The results (Fig. 1A) show that all strains contain at least one copy of a nis gene. From previous studies (26, 27) and the complete nucleotide sequence analysis of the 10-kb nis operon of Tn5276 (18, 35), we know that the nisA gene is located on a 4.2-kb HindIII fragment that is flanked by parts of IS1068 (26, 27) and the nisT gene (18). In nisin A producers, this part of the nis operon is very well conserved at the sequence level, as was found by comparing the Tn5276 nis genes and their upstream region with those of Tn5301 (15), Tn5307 (32), and L. lactis 6F3 (5). This explains why all nisin A-producing strains contained an approximately 4.2-kb HindIII fragment that hybridized with the nis probe. However, the nisin Z producers contained HindIII fragments of either 4.7 kb (NIZO 22186) or approximately 9.6 kb (other strains) that hybridized to the nis probe (Fig. 1A), suggesting that the organization of the region upstream of the nisZ gene is different from that preceding the nisA gene.

**Distribution of IS1068-like elements and characterization of IS1069 preceding the nisZ gene in L. lactis NIZO 22186.** To study the organization upstream of the nisZ gene in the nisin Z
FIG. 2. Architecture of left-end regions of nisin-sucrose elements and location of the probes used. The location of the iso-IS1086 elements (IS) is shown by the hatched block, and the arrowhead indicates the orientation of the putative transposase gene. The different classes are indicated. The boundary of left ends in the class III elements in ILC strains 19, 26 and pSL20, which contain a nisZ gene but do not hybridize with the IS-I, IS-II, or Tn5276-L probes, has not been determined, as is indicated by the dotted lines. The sizes of the hybridizing fragments are taken from Fig. 1. —, no hybridization or hybridization to HindIII fragments that do not contain the nisA or nisZ gene; X, hybridization to a HindIII fragment which varies in size between strains of one class because of the variable location of the chromosomal HindIII site marked with an asterisk. H, HindIII site.

producers, we first determined whether they contained an IS1068-like element by hybridization of their DNAs with the IS-I probe, which is specific for iso-IS1068 elements and is located on the 4.2-kb HindIII fragment which also contains the nisA gene in Tn5276 and Tn5301 (4, 26, 27) (Fig. 2). Similar experiments were done with the nisin A producers. The results (Fig. 1B) showed that IS1068-like elements are abundant in most strains and that they could potentially be used as a way to differentiate strains by IS typing, as suggested previously (9). Only the nisin A producers appeared to contain an iso-IS1068 element upstream of the nisA gene, since the same 4.2-kb HindIII fragment hybridized to the nis probe and the IS-I probe. However, to detect inverted IS1068-like elements upstream of the nis genes, the same digested DNAs were also hybridized to the IS1068-specific probe IS-II, which is complementary to sequences between the HindIII site in IS1068 or IS904 and the left end of Tn5276 or Tn5301 (4, 26, 27) (Fig. 1). The results (summarized in Fig. 2) showed that such an inverted IS1068-like element was present only upstream of the nisZ gene in strain NIZO 22186.

To substantiate this finding, the nucleotide sequence of the region around the HindIII site upstream of the nisZ gene of L. lactis NIZO 22186 was determined (Fig. 3). Indeed, this region contained an IS-like element that was very similar to IS1068 from Tn5276. We have designated this element IS1069 (registered with the Plasmid Reference Centre [20]). As expected, IS1069 was in the opposite orientation from IS1068. More importantly, the nucleotide sequence of the region left of IS1069 was completely different from that of the corresponding region in NIZO R5, and only half of the TTAT target repeat that flanks IS904 in Tn5301 (4) and IS1068 in Tn5276 (26) was found to border IS1069 (Fig. 3B). Since this region constitutes the left end of Tn5276, we propose that IS-mediated rearrangements have dislocated or deleted the left end of the nisin-sucrose element of L. lactis NIZO 22186, which as a consequence has become defective, since conjugative excision, the first step of its conjugal transposition, would be impaired.

The here-characterized IS1069, located upstream of the nisZ gene in L. lactis NIZO 22186, is the fifth in a group of L. lactis IS-like elements that show high (94 to 99%) nucleotide sequence identity and belong to the IS3 group of insertion sequences (4, 16, 26). However, the small differences on the nucleotide level result in important differences in the coding capacities of these elements. IS1068 and IS1069, from Tn5276 and L. lactis NIZO 22186, respectively, show an organization of open reading frames (ORFs) that is similar to that found in most members of the IS3 group: a small ORF A potentially translationally coupled to a larger ORFB, together covering almost the entire IS element (Fig. 3A) (24). The ORFAB fusion protein is probably the active transposase, and production of the transposase as a fusion protein may play a role in the regulation of IS transposition activity (23). In IS904 from Tn5301, several deletions with respect to IS1068 and IS1069 result in the lack of coding capacity for the first 440 bp of the element, and only one ORF is present (4). This suggests that IS904 is no longer transpositionally active. IS1076L and IS1076R from L. lactis Z270 (16) possess one large ORF covering the region that encompasses ORFA and ORFB in IS1068. Regulation of the transposition activity of the latter IS elements apparently is not effected through production of the transposase as a fusion protein.

Relationship between the architecture of nisin-sucrose elements and their conjugative capacity. The results concerning the organization of the nisin-sucrose element in NIZO 22186 suggest that in other nonconjugal strains, rearrangements in the region upstream of the nisin structural gene or elsewhere
have rendered the nisin-sucrose elements nonconjugative. To test this possibility, we studied the architecture of nisin-sucrose elements with probes specific for the left and right ends of Tn5276 (Fig. 1C and 1D) in the eight conjugative and the five nonconjugative nisin producers. The results (summarized in Table 1 and Fig. 2) showed that three classes of nisin-sucrose elements could be distinguished, designated classes I, II, and III. Class I (or the Tn5276-like class) includes elements that contain a nisA gene and a left end region (including an iso-IS1068 element) that is similar or identical to that of Tn5276 from strain NIZO R5. All elements in this class are conjugative except that of L. lactis INRA 3. The class II

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**Fig. 3.** Nucleotide sequence of IS1069 and its surrounding region. (A) Nucleotide sequence of IS1069. The amino acid sequences deduced from the ORFs of significant length present in the sequence are given below the nucleotide sequence. Potential ribosome-binding sites are denoted by stars over the bases that are complementary to the 3' end of L. lactis 16S rRNA (21). The HindIII site is in boldface type. A putative −10 TATAAT box is underlined, as are the 32-bp imperfect inverted repeats of the IS element and the potential frameshift promoting motif (AAAAAAAG [24]). The amino acid numbering corresponds to ORFAB, generated by a frameshift similar to that found for IS911 (23). Nucleotides in the DNA sequence and amino acids in the deduced ORFs that are different in IS1068 are shown above the nucleotide sequence and below the amino acid sequence, respectively (amino acid differences are also boxed). (B) Nucleotide sequences of the regions flanking IS1068 in Tn5276 and IS1069 in L. lactis NIZO 22186. The IS element sequence is in boldface type. The 4-bp target repeat flanking IS1068 is underlined.
elements (or the Tn5276-like elements) contain a nisZ gene and do not contain a sequence that hybridizes to the IS1068 probes immediately upstream of this gene. However, elements of this class do contain a region that is similar or identical to the left end of Tn5276 upstream of the nisZ gene, and all of them are conjugative. The class II element-containing strain NCK400 is completely devoid of IS1068-like sequences. This shows that iso-IS1068 elements are not involved in the conjugative transposition of nisin-sucrose elements. The elements in class III all contain a nisZ gene, but this is not immediately preceded by a sequence hybridizing to the Tn5276 left-end probe. This is compatible with the finding that all class III elements are nonconjugative.

There are two groups of class III elements (Fig. 2). Strains ILC19, ILC126, and ILCpSL20 belong to one class and completely lack sequences with homology to the left end of Tn5276 (Fig. 1C). These strains also contain no IS1068-like element immediately preceding the nisZ gene, as was found in the hybridization studies (Fig. 1B) and by PCR amplification. In the latter experiments, amplification was sought with IS-I or IS-II as a left-end primer and a primer located downstream of the nisin structural gene (nis10) as a right-end primer. With DNA from strains NIZO R5 and NIZO 22186, in which the distance between the IS element and the nis gene is approximately 750 bp, amplification products of the expected sizes were obtained (NIZO R5, 1.2-kb product with IS-I and nis10; NIZO 22186, 1.2-kb product with IS-II and nis10), while DNA from strains ILC19, ILCpSL20, and ILC126 did not yield any products with either primer combination (data not shown). Since the limit of the PCR amplification reaction in our hands is approximately 2.5 kb, these results indicate that there is no iso-IS1068 element present in approximately 2 kb of the region upstream from the nisZ gene in the DNA of strains ILC19, ILCpSL20, and ILC126. Strain NIZO 22186 is the only representative of the other group of class III elements (Fig. 2). It contains sequences that hybridize to the left end of Tn5276, but these are not located at the appropriate position upstream of IS1069 and the nisZ gene. This supports the assumption presented above that an iso-IS1068-mediated rearrangement has occurred in strain NIZO 22186. This rearrangement might be an inversion of the region between two inversely oriented IS elements or deletion of the region between two directly repeated IS elements through homologous recombination. Alternatively, IS1069 might have inserted as part of a composite transposon.

All strains were found to contain a region(s) that is similar or identical to the right end of Tn5276. Thus, all nonconjugative elements except for that of strain INRA 3 differ from conjugative elements by lacking a sequence that is similar to the Tn5276 left end at the appropriate location. As proposed for strain NIZO 22186, the absence of the left end presumably prevents excision and thereby conjugal transfer. Apparently, other causes underlie the inability of strain INRA 3 to conjugally transfer sucrose proficiency.

Evolution of nisin-sucrose elements in L. lactis. Class II nisin-sucrose elements are conjugative transposons encodong nisin Z production. Since they do not contain iso-IS1068 elements upstream of their nisZ genes (Fig. 1), it is likely that these class II elements are closest to the original nisin-sucrose element. The G+C content of IS1068 (37.7% [25]) is close to the L. lactis G+C content of approximately 38% (17, 34), while the G+C content of the region surrounding the IS element and of other regions of Tn5276 averages 30% (25). This suggests that IS1068 was not originally part of the transposon and that the transposon originated outside the genus Lactococcus. The nisin Z producer L. lactis NCK400, which was isolated from vegetables (14) and is completely devoid of iso-IS1068 elements, could be a primary lactococcal source of an original nisin-sucrose element, which then would belong to class II. The class I element would then have been generated from a class II element by insertion of an iso-IS1068 element, resulting in elements such as Tn5276. Since all class I elements contain a nisA gene, a nucleotide substitution resulting in nisin A production must have also occurred early in the generation of the class I element. Elements of classes I and II were able to spread themselves into different strains. Class III elements appear to be descendants of class II elements that were fixed in their positions by rearrangements that dislocated or deleted the left end of the transposon. Thus, class III elements are no longer transposons.

Unknown genetic elements related to Tn5276 might be present in the genomes of several L. lactis strains. In the DNA of some strains, more than one band hybridized to the Tn5276-L and -R probes (Fig. 1C and D). Strain ILCpSL5 (Fig. 1, lanes 11) apparently contains two copies of its class II nisin-sucrose element. Two HindIII fragments hybridized to the Tn5276-L probe (Fig. 1C), and because there is no IS1068-like element between the left end and the nisZ gene in class II elements, these fragments also hybridized to the nisZ probe (Fig. 1A). The hybridization to the Tn5276-R probe showed two strong signals, probably corresponding to the right ends of the two class II elements. In addition to these strong hybridization signals, two additional signals of lower intensity could be seen in the hybridizations with the left- and right-end probes. A possible explanation for this could be the presence of two genetic elements with ends that are similar to those of Tn5276.

In several other strains, additional weak or even strong hybridizations with the Tn5276-L probe and/or the Tn5276-R probe were observed (Fig. 1C and D). IS-mediated duplications could be responsible for the occurrence of additional hybridizing fragments. Alternatively, the reason for strong additional hybridizing fragments could be the presence of genetic elements that contain regions similar to the left or right end of Tn5276. Preliminary experiments with L. lactis MG1614 indicate that a region that is very similar to the right end of Tn5276 is present in this strain, including the genes responsible for excision (25). For further knowledge of gene transfer and evolution in L. lactis, it would be of interest to identify this and other cryptic elements that show similarity to parts of Tn5276.

Practical implications. Conjugal strategies have been applied to generate novel lactococcal strains that can be used in industrial milk fermentations (for a recent review, see reference 10). Even before the description of nisin-sucrose conjugative transposons, this approach has been used to generate starter strains that produce nisin A (1, 8). The results presented in this article show that there are two classes of nisin-sucrose elements (class II and class III) that encode the production of nisin Z. The observation that class II elements are conjugative now allows the construction of industrial starter strains that produce nisin Z, which has better diffusion properties than nisin A (3).

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