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Transposition in *Lactobacillus sake* and Its Abolition of Lactocin S Production by Insertion of IS*1163*, a New Member of the IS*3* Family

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This report presents the nucleotide sequence and insertional activity of IS*1163*, which is a new member of the IS*3* family of transposable elements. Analysis of spontaneous mutants of the lactocin S-producing *Lactobacillus sake* strain L45 show that the bacteriocin-negative phenotype is due to either loss of the producer plasmid or the insertion of IS*1163* into the lactocin S operon (*las* operon). The data further show that insertional inactivation of the lactocin S operon is the result of a transposition event involving a chromosomally located donor copy of IS*1163*. Although the insertions described are clustered within a 250-bp region of the *las* operon, there are no features of the insertion sites to suggest target-specific insertion of IS*1163*. The overlapping, frameshifted organization of the two major open reading frames found in IS*1163* is typical for the IS*3* family, but the structure of the putative frameshift region includes features which distinguish IS*1163* from the other members of the group. The insertional activity of IS*1163* in *L. sake* L45 has aided in identifying regions of pCIM1 essential for lactocin S production and may have further practical applications as a mutational tool in *L. sake*.

In the last few years, a number of bacteriocins from lactic acid bacteria have been isolated and characterized. The lactic acid bacterial bacteriocins constitute a group of peptide antagonists which are generally small (typically below 5 kDa) and which are processed from ribosomally synthesized precursors. The group is therefore considered to be distinct from the typically high-molecular-weight bacteriocins of gram-negative organisms and from peptide antibiotics like gramicidin S, which are assembled by multienzyme complexes (11).

The bacteriocin lactocin S was originally described as an antimicrobial activity produced by *Lactobacillus sake* L45 (13) and was later found (14, 15, 27) to belong to the lantibiotic subgroup of bacteriocins (2, 9). *L. sake* L45 contains two plasmids, pCIM1 and pCIM2, with molecular sizes of approximately 50 and 34 kb, respectively. While no traits have yet been assigned to pCIM2, it has been shown that lactocin S production depends on the presence of pCIM1 (13). The lactocin S-producing phenotype of L45 is unstable; nonproducers arise with a frequency of approximately 3/1,000 CFU in an overnight culture of L45, as judged by a simple overlay assay (13). All the isolated mutants are stable and do not differ significantly from the original producer strain in growth characteristics (13, 27).

The lactocin S production mutants were categorized according to plasmid content and immunity phenotype (13), and the isolates that have lost both pCIM1 and immunity (Bac^- Imm^-) constitute the largest group. Some of the nonproducers, however, retain both immunity and, apparently, pCIM1. Mutants belonging to this category arise with an approximate frequency of 1/1,000 CFU in an overnight culture of L45 (13, 27), and analyses show that the Bac^- Imm^+ phenotype is correlated with an altered restriction pattern in plasmid digests. This suggests that the abolition of lactocin S production is caused by DNA rearrangement in these cases, and the high frequency of occurrence indicates the involvement of a transposable element. Knowledge of insertion sequence activities stems almost exclusively from a small number of elements isolated from gram-negative organisms, and only one insertion element from the genus *Lactobacillus* has been characterized in any detail previously (25, 26).

This report presents the nucleotide sequence and some of the basic transpositional activities of IS*1163*, which is a new IS*3*-related element of gram-positive origin. The results also offer an explanation for the frequent cessation of lactocin S production in *L. sake* L45, which was the primary goal of the study.

MATERIALS AND METHODS

Bacterial strains and plasmids. *L. sake* strains and plasmids used in this study are listed in Table 1. The strains were

TABLE 1. *L. sake* strains and plasmids described in this study

Strain or plasmid[a]	Plasmid content or structure	Relevant phenotype
L. sake strains		
L45	pCIM1, pCIM2	Bac^+ Imm^+
L45-2.1	pCIM1	Bac^+ Imm^+
L45-3.1	pCIM2	Bac^- Imm^-
L45-4.1	pCIM141, pCIM2	Bac^- Imm^+
L45-4.2	pCIM142, pCIM2	Bac^- Imm^+
L45-5.1	pCIM151	Bac^- Imm^+
Plasmids		
pCIM1		Bac^+ Imm^+
pCIM141	pCIM1 (*lasM1*::IS*1163*)	Bac^- Imm^+
pCIM142	pCIM1 (*lasM2*::IS*1163*)	Bac^- Imm^+
pCIM151	pCIM1 (*lasM3*::IS*1163*)	Bac^- Imm^+
pCIM2		Cryptic

[a] The designations correspond to the categories of lactocin S mutants described previously (13).

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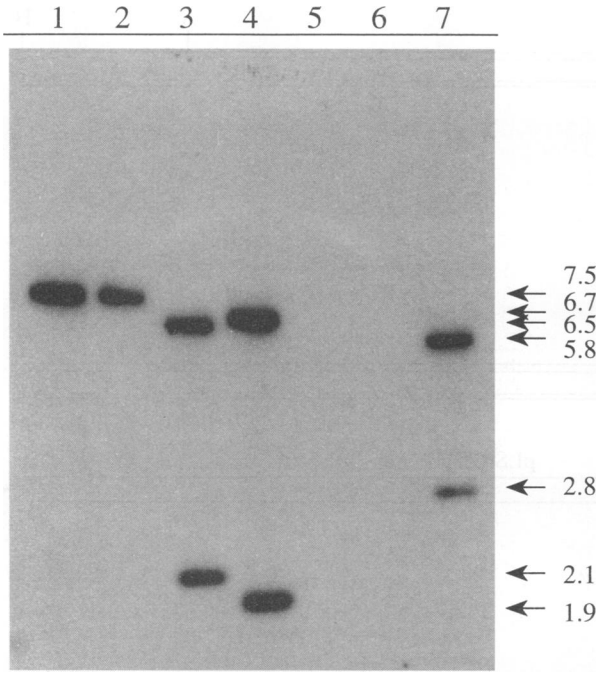

FIG. 1. Southern analysis showing the correlation between restriction pattern and Bac phenotype. *Hin*dIII-digested plasmid DNA from the different isolates was probed with a 7.5-kb fragment containing the sequenced portion of the *las* operon (26). Lane 1, L45; lane 2, L45-2.1; lane 3, L45-4.2; lane 4, L45-4.1; lanes 5 and 6, L45-3.1; lane 7, L45-5.1. The numbered arrows indicate the sizes (in kilobases) of the hybridizing fragments.

cultivated at 30°C in MRS (Difco) liquid medium without aeration or on MRS agar (1.5%).

Escherichia coli DH5α (7) was cultivated at 37°C in Luria-Bertani liquid medium with aeration or on Luria-Bertani agar. Ampicillin for selecting and propagating resistant bacteria was added to a final concentration of 50 μg/ml.

The plasmids pLS4.2E, pLS4.2HE, and pLS4.2H are described in the text and in Fig. 2. Plasmids were isolated by the standard alkaline lysis method (21), with elevated lysozyme concentrations (final concentration, 5 mg/ml) when plasmids from *L. sake* were isolated. Total DNA was isolated from cultures of *L. sake* by the following procedure: after initial lysozyme treatment (5 mg/ml in 100 mM Tris-Cl [pH 8.0]–10 mM EDTA) at 37°C for 10 min, proteinase K (final concentration, 100 μg/ml) and sodium dodecyl sulfate (SDS; final concentration, 0.5%) were added. The lysate was incubated for 1 h at 37°C and subsequently extracted once with chloroform (chloroform-isoamyl alcohol, 24:1), twice with phenol (saturated with Tris-EDTA, pH 8.0), and once with phenol-chloroform. NaCl was added to 0.4 M, and the nucleic acid was precipitated with 2.5 volumes of ice-cold ethanol. After a wash in 70% ethanol, the nucleic acid was dissolved in Tris-EDTA, pH 8.0, containing 50 μg of RNase A per ml.

Molecular cloning. Enzymes used in the cloning experiments were purchased from New England Biolabs Inc., Promega Corp., or Boehringer Mannheim GmbH. The pUC18 plasmid was purchased from Pharmacia; pGEM7Z f(+) was purchased from Promega Corp.

E. coli DH5α was transformed by electroporation (4) using the Bio-Rad Gene Pulser.

Amplification (PCR) and nucleotide sequence determination. DNA was amplified in 100-μl reaction mixtures (2.5% formamide, 1 μM concentrations of each of the primers, 1× reaction buffer, 200 μM concentrations of each of the deoxynucleoside triphosphates, 1 μl of template DNA [0.1 to 10 ng], and 2.5 U of AmpliTaq polymerase) under mineral oil with a Perkin-Elmer N801-0177 DNA Thermal Cycler or a model PTC-150 Minicycler (MJ Research). Amplification typically proceeded through 30 cycles after a 3-min hot start (97°C). The polymerizing time (72°C) was set to 1 min 30 s in all reactions except during amplification from ligation mixes, when the polymerizing time was set to 3 min. The annealing temperature was set at the T_m value for the oligonucleotide having the lowest calculated melting point. Annealing time and denaturing time (94°C) were set to 1 min in all reactions.

Sequenase (U.S. Biochemicals) was used in all sequencing reactions. Double-stranded plasmid templates were prepared for sequencing as described by Kraft et al. (12). Amplified DNA was sequenced either directly after removal of primers and heat denaturation or after strand separation using magnetic beads (Dynabeads M-280 Streptavidin; Dynal). Primers used for amplification and sequencing except for the pUC/M13 universal primers (Promega Corp.) were synthesized on an Applied Biosystems model 318A DNA synthesizer. For sequencing IS*1163* with the subclones generated from pCIM142, a primer-walking strategy was employed: initially, sequence data were obtained with pUC/M13 universal primers and primers used in the sequencing of the 7.5-kb *Hin*dIII fragment of pCIM1 (27). The sequence information obtained from these reactions was used to design primers for the next round of sequencing, and the procedure was repeated until contiguous sequences had been generated in both directions.

DNA-DNA hybridization. Restricted DNA was separated on agarose gels and blotted to GeneScreen Plus membranes (DuPont) with the Vacugene blotting apparatus (LKB Pharmacia). Hybridization probes were labeled with the Boehringer Mannheim Random Primed DNA Labeling Kit and [α-^{32}P] dATP (3,000 Ci/mmol; Amersham). Prehybridization and hybridization conditions were 0.5 M Na-phosphate (pH 7.2), 7% SDS, and 65°C. The membranes were prehybridized in 300 ml for 30 min in a shaking water bath and transferred to a bottle containing 10 to 20 ml of preheated prehybridization solution before the denatured probe was added. Hybridization proceeded in a Hybaid hybridization oven overnight. The membranes were washed (300 to 500 ml, 20 min in a shaking water bath) once in 100 mM Na-phosphate (pH 7.2)–1% SDS at room temperature, twice in the same buffer at 65°C, and twice in 50 mM Na-phosphate (pH 7.2)–1% SDS at 65°C. Autoradiography was carried out with Cronex film (DuPont) and an intensifying screen at −85°C.

Nucleotide sequence analysis. Nucleotide sequence data were analyzed using PC-Gene (Intelligenetics, release 6.5) running on an IBM PC or the University of Wisconsin Genetics Computer Group programs, version 7.2, running on a VAX mainframe computer.

Nucleotide accession number. The IS*1163* nucleotide sequence has been assigned accession number X75164 in the EMBL data library.

RESULTS

Identification and sequence determination of plasmid-inserted IS*1163*. *Hin*dIII-restricted plasmid DNA from the original producer strain and six other isolates differing in plasmid profiles and/or Bac Imm phenotype was subjected to Southern

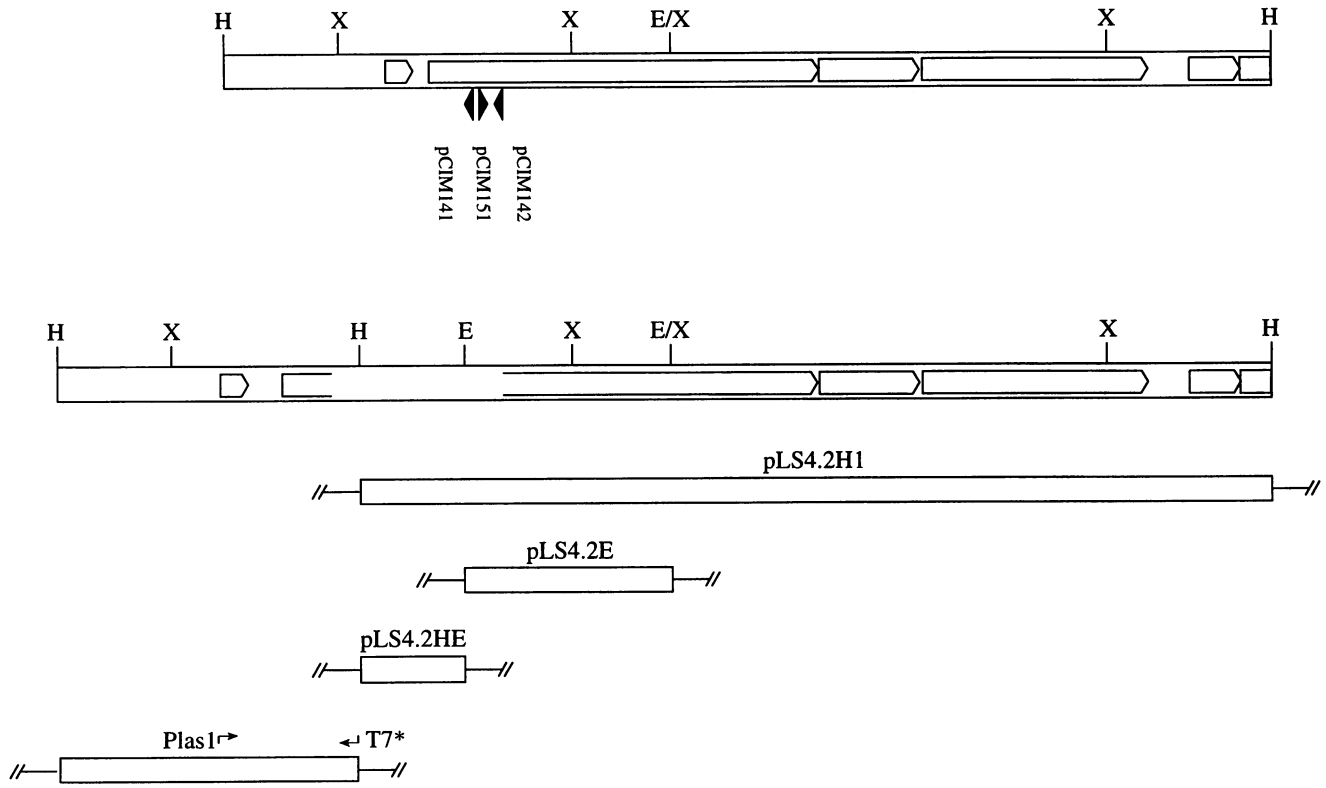

FIG. 2. Cloning and sequencing strategy employed in the characterization of IS*1163* and the positions of the IS*1163* insertions associated with the Bac$^-$ Imm$^+$ phenotype. The localization of the *las* operon within the 7.5-kb *Hin*dIII fragment of pCIM1 is shown in the upper part of the figure, with black triangles indicating the positions of the insertions giving rise to the mutant plasmids. The corresponding region in plasmid pCIM142 and the strategy employed to determine the IS*1163* sequence are shown below. The open boxes represent the three cloned and sequenced fragments of pCIM142 and the fragment from which the rightmost part of the insertion was amplified and sequenced. Plas1 denotes the *las*-specific primer used in the amplification reaction, while E, H, and X represent sites for the restriction enzymes *Eco*RI, *Hin*dIII, and *Xba*I, respectively.

hybridization analysis using a 7.5-kb *Hin*dIII fragment from pCIM1 containing (part of) the lactocin S operon (27) as a probe. This fragment was absent in the *Hin*dIII digests of plasmids isolated from the Bac$^-$ isolates. The result of the hybridization experiment is shown in Fig. 1. No hybridization signal was obtained from the isolates that had lost pCIM1, and the two producing strains, L45 and L45-2.1, both gave one signal corresponding to the 7.5-kb probe, as expected. Each of the Bac$^-$ Imm$^+$ variants had two hybridizing fragments, and the sum of fragment sizes for any of these isolates was approximately 8.6 kb. To explain these hybridization patterns, the insertion of 1.1 to 1.2 kb of DNA containing a *Hin*dIII site at different locations and/or different orientations within the 7.5-kb fragment was proposed. The strategy employed to test the insertion hypothesis is outlined in Fig. 2.

The 6.5-kb fragment from pCIM142 hybridizing to the 7.5-kb pCIM1 fragment was ligated to pUC18 and cloned in *E. coli*, giving rise to plasmid pLS4.2H. The 6.5-kb fragment was subsequently isolated and treated with *Eco*RI, and the two smallest of the three fragments thus generated were subcloned and sequenced. The left junction between pCIM1 and the inserted DNA was sequenced after amplification: a *Hin*dIII digest of pCIM142 was ligated to pGEM7Zf(+), and the junction region was amplified with a primer derived from the 7.5-kb *Hin*dIII fragment and a biotinylated primer complementary to the T7 promoter sequence of the vector (T7*). The complete sequence of IS*1163*, which has a GC content of 39%, is shown in Fig. 3.

To determine the points of IS*1163* insertion in pCIM141 and pCIM151, plasmid DNA was digested to completion with *Sau*3AI and ligated to *Bam*HI-digested pGEM7Zf(+). The junction sequences were amplified from the ligation products with a biotinylated vector-specific primer (T7*; see above) and a primer specific to either end of IS*1163*. The localization and orientation of the inserted element were subsequently determined by aligning the junction sequences with the pCIM1 sequence.

Genomic distribution of IS*1163*. In order to determine the genomic copy number of IS*1163* in the various isolates, the element was amplified from pCIM142, labeled, and used to probe a Southern blot of *Xba*I-digested plasmid and total DNA from the various isolates. This enzyme has three sites in the 7.5-kb *Hin*dIII fragment of pCIM1 but does not cut IS*1163* (Fig. 2). The hybridization result is shown in Fig. 4. Two hybridizing fragments with molecular sizes of 14 and 6.5 kb were detected in the total DNA preparations only and thus represent chromosomal copies of IS*1163*. The hybridization results suggest that the chromosomal copies have the same localizations in all the isolates. As expected from the restriction map of the 7.5-kb *Hin*dIII fragment (Fig. 2), a 2.7-kb hybridizing fragment is present in both plasmid and total DNA preparations from the Bac$^-$ Imm$^+$ variants but is absent from the lactocin S-producing and Bac$^-$ Imm$^-$ isolates.

To determine the flanking sequences of the chromosomally located copies of IS*1163*, the same strategy used when plasmid insertion points were determined was followed: L45 total DNA

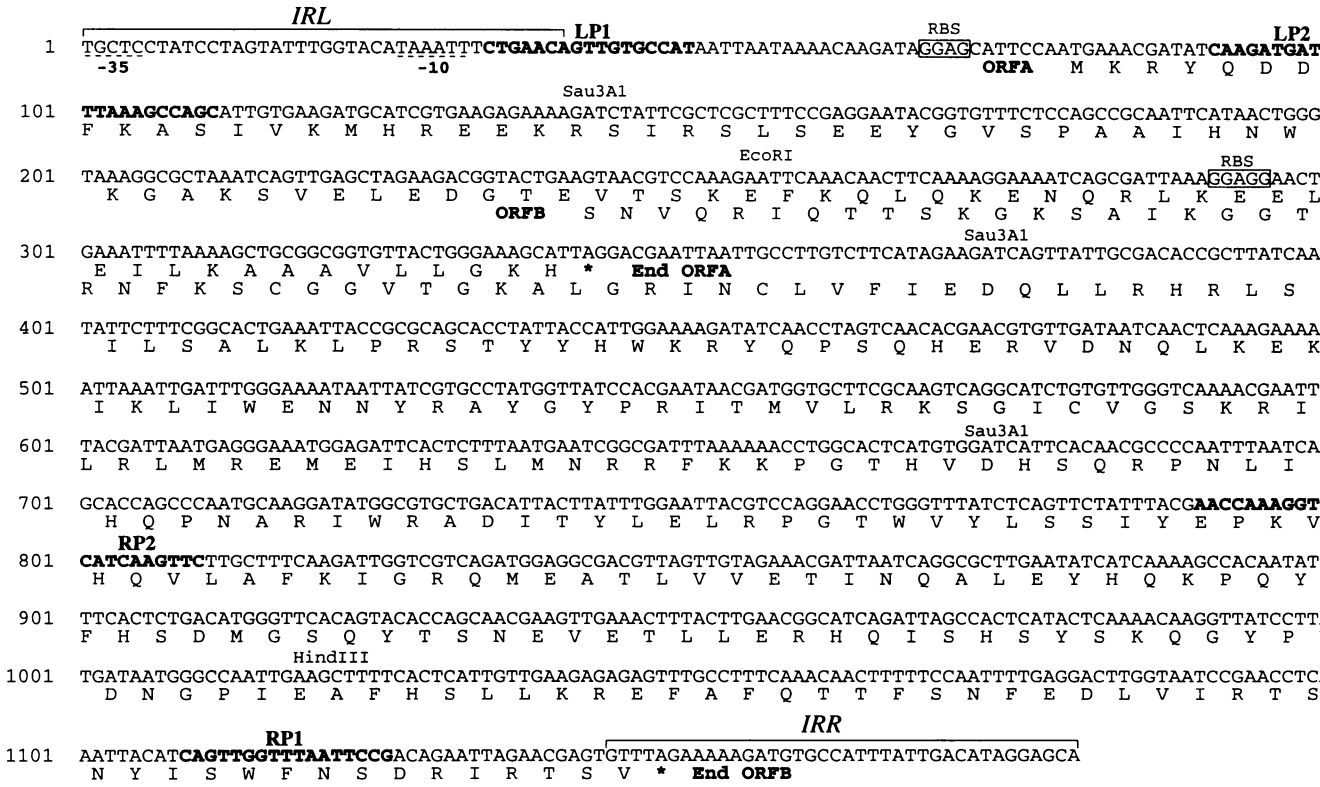

FIG. 3. Nucleotide sequence of IS*1163*, with the ORFA and ORFB translations shown below the coding sequence. Putative ribosome binding sites (RBS) are boxed, and the suggested promoter region is indicated in boldface below the sequence. The left and right terminal inverted repeats (IRL and IRR, respectively) are marked by brackets, and the sites of the relevant restriction endonucleases are indicated above the sequence. LP1, LP2, RP1, and RP2 designate the sequences (shown in boldface) corresponding to the oligonucleotide primers used for amplification and sequencing of the regions flanking the IS*1163* insertions. For LP1 and LP2, the actual oligonucleotide sequences are complementary to the ones indicated in the figure.

was digested to completion with *Sau*3AI and ligated to *Bam*HI-digested pGEM7Zf(+). The ligation mix served as a template for two separate amplification reactions, using the biotinylated vector-specific primer (T7*) and a primer specific for either the left end or the right end of IS*1163*. Biotinylated PCR products were purified from the reaction mixtures, and the strands were separated and sequenced as described in Materials and Methods. There were no significant background signals on the sequencing gels, allowing unambiguous sequence determination. In order to establish whether these junction sequences were associated with the same IS*1163* copy, amplification reactions were set up with primers derived from the sequences shown in Fig. 5A on total DNA from the different isolates. A single PCR product was obtained from all the reactions (Fig. 5B), showing that the two flanking sequences are associated with the same copy of IS*1163* and that this insertion point is preserved among the isolates tested. The PCR product of the L45 reaction was sequenced and found to be identical to the sequence presented in Fig. 3.

Sequence analysis of IS*1163*. IS*1163* is a typical bacterial insertion sequence, with respect to both size and genetic organization (6). The total sequence of 1,180 bp includes terminal inverted repeats of 39 bp (28 of 39 nucleotides identical) and two overlapping open reading frames (ORFA and ORFB). The overlap between ORFA and ORFB is 100 bp, and the reading phases are 0 and −1, respectively. The result of a search for promoter sequences in the IS*1163* sequence indicates that transcription of the ORFA-ORFB unit may be initiated at the extreme left end of the element (Fig. 3). The putative −35 box lacks a 5′ T to fit the consensus sequence, but the target DNA completes the putative IS*1163* promoter element in both the chromosomal and the pCIM142 insertions. The spacing between the −10 and −35 hexanucleotides is somewhat larger (20 bp) than expected for an efficient promoter (20). The presence of a perfect −35 box hexanucleotide (5′-TTGACA-3′, nucleotides 1167 to 1172) in the right inverted terminal repeat suggests that IS*1163* participates in the generation of a hybrid promoter upon insertion, although analysis of the junction sequences created by the insertions failed to identify any such promoters. No probable transcriptional terminator structure was found within the IS*1163* sequence.

The AUG suggested (positions 79 to 81, Fig. 3) as the initiation codon of ORFA is situated 7 nucleotides downstream from a sequence matching the consensus bacterial ribosome binding site. For ORFB, however, it is difficult to point out the most probable point of translation initiation; there are no combinations of a standard initiation codon and a proper ribosome binding site sequence associated with ORFB.

A number of insertion sequences related to IS*1163*, most of which belong to the IS*3* family of transposable elements (22), were identified by database searches using the translations of the IS*1163* ORFs as probes. Alignments of ORFA and ORFB

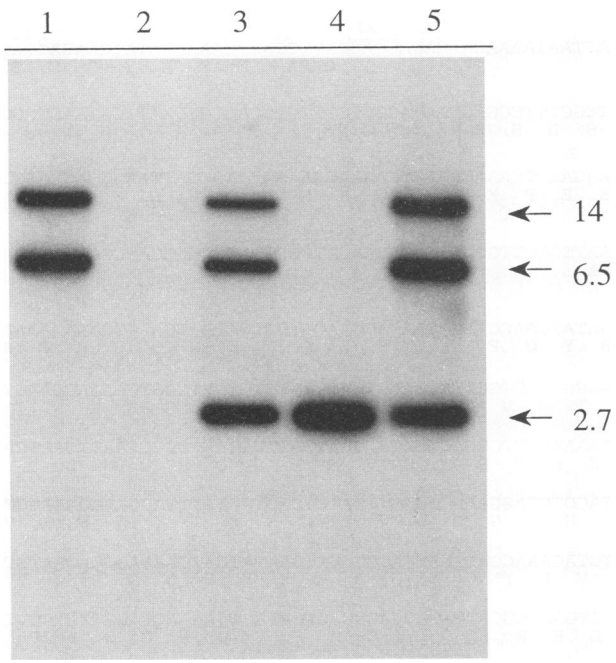

FIG. 4. Southern hybridization showing the genomic distribution of IS*1163* in L45 and some of its derivatives. Total and plasmid DNAs were restricted with *Xba*I, transferred to a membrane, and probed with IS*1163* DNA amplified from pCIM142 (see also Fig. 5). Lane 1, L45 total DNA; lane 2, L45 plasmid DNA; lane 3, L45-4.1 total DNA; lane 4, L45-4.1 plasmid DNA; lane 5, L45-4.2 total DNA. The numbered arrows indicate the sizes (in kilobases) of the hybridizing fragments.

with the corresponding ORFs of some of the related elements are shown in Fig. 6.

A common feature of elements in the IS*3* family is the overlapping, out-of-phase organization of the two major ORFs encoding the transposase. It has been demonstrated that a frameshift event fusing the products of the two reading frames is essential for transposase activity (16, 28). The region of IS*1163* shown in Fig. 7 is arranged to emphasize the similarity between IS*1163* and IS*911* with respect to features proven or proposed to be of significance to frameshifting in the latter case (3, 16).

All the IS*1163* plasmid insertions described occur within a 250-bp region of the *las* operon (Fig. 2). Despite this clustering of insertions, however, there are no similarities between the insertion sites to suggest any target specificity. All the IS*1163* insertions examined are flanked by 3-bp direct repeats, but the duplicated trinucleotide is different in each case (Fig. 5A).

DISCUSSION

Genetic effects of IS*1163* transposition. Immune nonproducers (Bac^- Imm^+) can be isolated from an *L. sake* L45 culture at a frequency of approximately 1/1,000 CFU (13, 27). The results presented here show that for the three Bac^- Imm^+ isolates examined, there is a correlation between the presence of a copy of IS*1163* in the second ORF of the lactocin S (*las*) operon and the abolition of lactocin S production, and we therefore conclude that IS*1163* insertion causes the cessation of production in these cases.

The simplest explanation for the observed inactivation of the *las* operon is that insertion results in aborted translation of the target ORF, which is presumably essential for lactocin S production. Transcription initiated from the putative IS*1163* promoter may also affect expression of the *las* operon.

Genomic distribution and mechanism of transposition. The hybridization data suggest the presence of two copies of IS*1163* in the L45 chromosome, while junction sequences could be amplified from only one chromosomally located copy. This could reflect the presence of only one bona fide copy of the element and one pseudocopy, assuming that the latter is mutated to an extent preventing adequate primer binding but allowing detection by hybridization. However, the absence of amplified junction sequences from the second chromosomal copy of IS*1163* may be a consequence of experimental limitations; i.e., the stretch of DNA separating the primers may have been too long to allow quantitative amplification under the conditions chosen.

The insertional inactivation of lactocin S production is obviously the result of a transposition event involving a chromosomally located copy of IS*1163*. The mechanism by which the element transposes is unclear, however: although the copy number increase associated with insertion into pCIM1 suggests a replicative mode of transposition, the data are also consistent with a nonreplicative event followed by segregation of the donor molecule.

The high frequency of IS*1163* insertions into pCIM1 may reflect a generally high frequency of transposition, or it may be the consequence of transposition into a preferred target. The insertion sites identified have no features to suggest any target specificity of insertion, although this specificity could be of a regional kind, which is not easily identified by sequence analysis (6). It is worth noting in this context that the G+C content of the DNA flanking the chromosomally located copy of IS*1163* is as low as 28%, which is close to the corresponding value for the sequenced region of pCIM1 (29.8%) but far from the expected genomic value of 43% for *L. sake* (10).

IS*1163* is a new member of the IS*3* family. Overall sequence similarity identifies IS*1163* as a member of the IS*3* family of bacterial insertion sequences (22). Expression of the transposase activity has been studied in detail for two of these elements, IS*911* and IS*150*, and it has been shown that transposition activity depends on a frameshift event which fuses the products of the overlapping reading frames (16, 17, 28). Translational frameshifting is also required for production of transposase in the otherwise unrelated IS*1* (23, 24). The dependence on frameshifting for the expression of essential genes is a feature that the IS*3* family shares with retroviruses as well as many bacterial and bacteriophage genes (1, 5), and the model for ribosomal rephasing was originally proposed to account for the presence of transframe proteins in retroviral systems (8). In the case of IS*911* and IS*150*, the efficiency of frameshifting is determined by the so-called tandem slippery codons (1), which is the heptanucleotide motif where the ribosomal rephasing occurs; the presence of a downstream secondary structure; and possibly an upstream Shine-Dalgarno site in the region where the two ORFs overlap (3, 16, 28).

The region of overlap between ORFA and ORFB of IS*1163* closely resembles corresponding regions in other members of the IS*3* family (Fig. 7). Structures in IS*1163* which may be of significance in the expression of the transposase genes are suggested by sequence alignments, and the IS*1163* frameshift window thus indicated has the sequence 5′-UUUAAAA-3′,

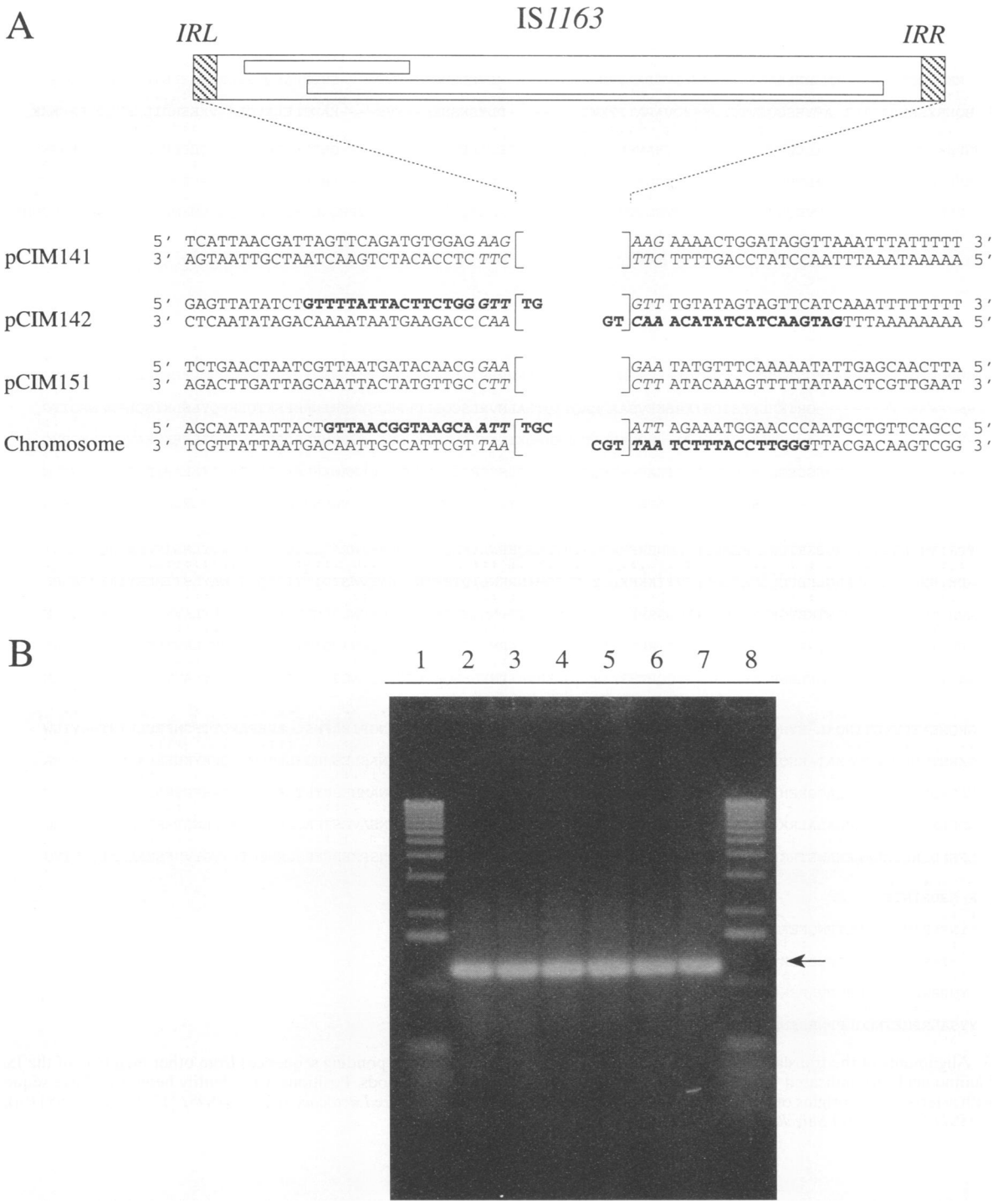

FIG. 5. (A) IS*1163* insertion points, showing the immediate flanking sequences of each insertion. The sequences corresponding to the oligonucleotides used for amplifying IS*1163* from pCIM142 and from the chromosomally located copy are shown in boldface, whereas the duplicated trinucleotides are italicized. (B) The products (arrow) after amplifying the chromosomally located copy of IS*1163* with the primers indicated above (bottom line). Lanes 1 and 8, molecular size markers (Bethesda Research Laboratories 1-kb ladder); lanes 2 and 7, L45; lane 3, L45-3.1; lane 4, L45-4.1; lane 5, L45-4.2; lane 6, L45-5.1.

which is novel in this context. Downstream of the frameshift window is a region which could form a stem-loop structure masking the stop codon of ORFA. This structure has analogs in both IS*911* (3, 16) and IS*150* (28), where a function in transposition activity has been demonstrated. A Shine-Dalgarno motif is situated upstream of the putative IS*1163* frameshift window, at a distance indicating a role in frameshifting and possibly in initiating translation of ORFB. The

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IS1163  MKRYQDDFKASIVKMHREEKRSIRSLSEEYGVSPAAIHNWVKG-A----KSVELEDGT--EVT----SKEFKQLQKENQRLKEELEILKAAAVLLGKH  (87)
IS981   MQKRYSKEFKETLI-AFYHSGQSVTQLSKEYDVAPATIYKWI--------DLYSKSNES--SVS----KADFLELKRQLAKVKEERDILKKVLTIFAEKKK  (86)
IS1068  MGRRKFDKQFKNSAVKLILEEGYSVKEVSQELEVHANSLYRWVQE-VEEYGESAFPGNGTALADA----QHKIKLLEKENRYLQEELELLKKFQVFLKRSK  (96)
IS1133  MSLKHSDEFKRDAVRIALTSGLTRRQVASDLSIGLSTLGKWIASIS----DETKIPTQD--TDL----LRENERLRKENRILREEREILKKAAIFFAVQKL  (91)
IS911   MKKRNFSAEFKRESAQLVVDQNYTVADAAKAMDVGLSTMTRWVKQ-L----RDERQGKTP-KASPITPEQIEIRELRKKLQRIEMENEILKKATALLMSDSLNSSR (100)
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B

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IS1163  SNVQRIQTTSKGKSAIKGGTRNFKSCGGVTGKALGRINCLVFIEDQLLRHRLSIILSALKLPRSTYYHWKRYQPSQHERVDNQLKEKIKLIWENNYRAYG  (100)
IS981   ----------------GRTRHLKKSIDHIRREKEVSAADMAQTIQTLALNVRLSCQLLDVPESSYYERINRHPSKTQLRRQYLSLKISQLFNANRGIYG  (83)
IS1068  --------------------TSKKVPGLLEA--KQVKRFEFLLKHHGKIKIKHAVKVLKVSRSGFYEYMHRRPSKQQVEREILSEKIKAVFHEHKGRYG  (77)
IS1133  -------TFTQREPYPSGGEGDIKKGSNIFRSTKAV--RFQFITDYRGSLSRSRICRLMGVTDRGLRAWKRRPPSLRQRRDLILLAHIREQHRLCLGSYG  (91)
IS911   ---------------------NIKKGYRALDVRLPEQ--FSIIGKLRAHYPVVTLCHVFGVHRSSYRYWKNRPEKPDGRR-AVLRSQVLELHGISHGSAG  (76)

IS1163  YPRITMVLRKSGICVGSKRILRLMREMEIHSLMNRRFKKPG-THVDHSQRPNLIKHQ----PNARIWRADITYLELRPGTWVYLSSIYEPKVHQVLAFKI  (195)
IS981   APKIHHLLLKQGEKVGLKLVQKLMKQLQLKSVVIKKFKPGY-SLSDHINRKNLIQTEPTK--KNKVWSTDITYIPTQQG-WAYLSTIMDRYTKKVIAWDL  (179)
IS1068  AVRITKVLHNTGIMTNTKRVGKLMHLMGLYAKGSRYKYKHYNRKGSSLSRPNLINQIFKATAPNKVWLGDMTYIPTKEGT-LYLAVNIDVFSRKIVGWSM  (176)
IS1133  RPRMTEELKALGLQVGQRRVGRLMRQNNITVVRTRKFKRTTDSHHTFNIAPNLLKQDFSASAPNQKWAGDITYVWTREG-WVYLAVILDLYSRRVIGWAT  (190)
IS911   ARSIATMATRRGYQMGRWLAGRLMKELGLVSCQQPTHRYKR-GGHEHVAIPNYLERQFAVTEPNQVWCGDVTYI-WTGKRWAYLAVVLDLFARKPVGWAM  (174)

IS1163  GRQMEATLVVETINQAL-EYHQKP--QYFHSDMGSQYTSNEVETLLERHQISHSYSKQGYPYDNGPIEAFHSLLKREFAFQTTFSNFEDLVIRTSNYISW  (292)
IS981   GKRMTVELVQRTLNKAI-KSQDYPEAVILHSDQGSQYTSLEYEELLKYYGMTHSFSRRGYPYHNASLESWHGHLKREWVYQFKYKNFEEAYQSIFWYIEA  (278)
IS1068  SSRMQDKLVRDYFLQACGKEHPQPG-LIVHTDQGSQYTSSRYQSTLRQVGAQSSMSRKGNPYDNAMMESFYKTLKRELINDAHFETRAEATQEIFKYIET  (275)
IS1133  GDRLKQDLALRALNMALALRKPPPG-CIQHTDRGSQYCAHEYQKLLLKHQLLPSMSGKGNCFDNSAVESFFKSLKAELIWRRHWQTRRDIEIAIFEYING  (289)
IS911   SFSPDSRLTMKALEMAWETRGK-PGGVMFHSDQGSHYTSRQFRQLLWRYQIRQSMSRRGNCWDNSPMERFFRSLKNEWIPVVGYVSFSEAAHAITDYIVG  (273)

IS1163  F-NSDRIRTSV                    (302)
IS981   FYNSKRIHQSLGYLTPNQFEKVSA        (302)
IS1068  YYNTKRMHSGLDYKSPKDFEKYNS        (299)
IS1133  FYNPRRRHSTLGWKSPVAFEKKAA        (313)
IS911   YYSALRPHEYNGGLPPNESENRYWKKL     (300)
```

FIG. 6. Alignments of the translations of ORFA (A) and ORFB (B) with the corresponding sequences from other members of the IS*3* family. Identical amino acids are indicated by colons; conserved changes are indicated by periods. Positions with identity between all five sequences are marked with asterisks. The origins of the elements compared with IS*1163* in the figure are *Lactococcus lactis* (IS*981* [12a] and IS*1068* [19]), *Erwinia amylovora* (IS*1133* [3a]), and *Shigella dysenteriae* (IS*911* [18]).

AUU codon, which has been shown to be the initiation codon of the IS*911* ORFB (16), is present also in IS*1163*, but in frame 0 rather than −1. The use of this codon for initiating translation of ORFB would require a −1 shift of reading phase immediately after initiation, an event which has been shown to be a prerequisite for translation of the second ORF (*insB*) of IS*150* (28). It should be noted at this point, however, that the identification of functional regions in IS*1163* lacks experimental support and that the significance of the features discussed above with respect to the expression of the transposase remains to be determined.

The results presented in this report show that IS*1163* is an active insertion sequence of the IS*3* family, which transposes with a high frequency to the plasmid-borne *las* operon in *L. sake* L45. The high insertional activity of IS*1163* transposition has already aided our investigation of the genes involved in lactocin S production, and the element may be of value as a mutational tool in *L. sake*. The insertional system described may also be used to address questions of a more general nature regarding the activities of this group of transposable elements in gram-positive organisms.

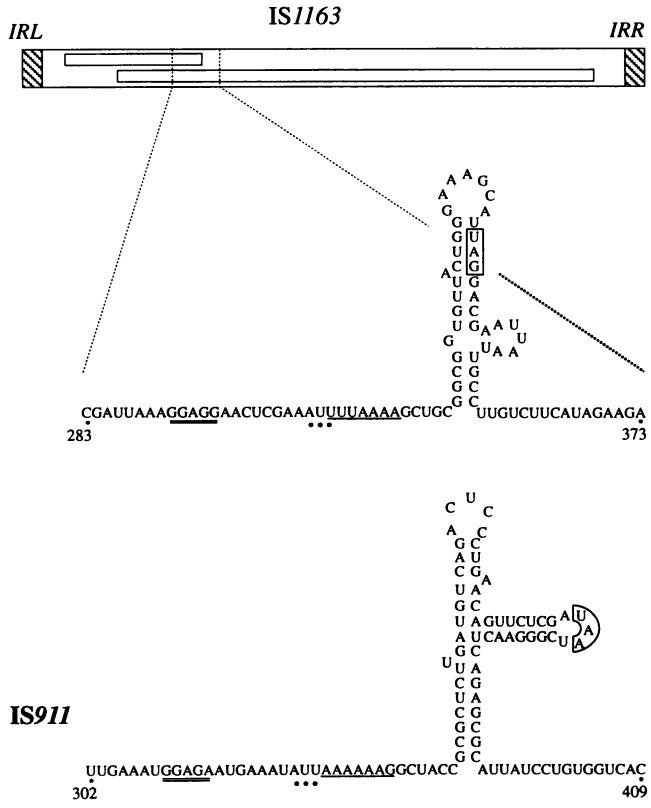

FIG. 7. Structure of IS*1163* and the putative frameshift region. The corresponding region of IS*911* is included for comparison. The tandem slippery sequences and Shine-Dalgarno motifs are indicated by single and double lines, respectively, and the ORFA stop codons are boxed. Bullets mark the AUU codons; that in IS*1163* is in the same reading frame as ORFA. Initiation of ORFB translation by this codon would therefore require a −1 frameshift immediately after initiation, which has been demonstrated for the related element IS*150* (28). In IS*911*, where it has been shown to be functional, the AUU codon is in the ORFB reading frame. Nucleotide coordinates are given with reference to the left termini of the elements. Note that for both IS*1163* and IS*911*, there is no experimental support for the particular stem-loop structure suggested; it represents but one of a number of alternative secondary structures of the mRNA.

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