APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1992, p. 1670–1676 Vol. 58, No. 5
0099-2240/92/051670-07$02.00/0

Conjugal Transfer in *Lactococcus lactis* of a 68-Kilobase-Pair Chromosomal Fragment Containing the Structural Gene for the Peptide Bacteriocin Nisin

T. GIREESH,[1] BARRIE E. DAVIDSON,[1] AND ALAN J. HILLIER[2]*

Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, Victoria 3052,[1] and Dairy Research Laboratory, Division of Food Processing, Commonwealth Scientific and Industrial Research Organisation, P.O. Box 20, Highett, Victoria 3190,[2] Australia

Received 7 August 1991/Accepted 12 February 1992

Nisin-producing transconjugants were generated by mating nisin-producing strains of *Lactococcus lactis* subsp. *lactis* with derivatives of *L. lactis* subsp. *lactis* LM0230. The sucrose-utilizing ability and reduced bacteriophage sensitivity were also transferred with the nisin-producing character. Pulsed-field gel electrophoretic analysis of genomic DNA from donor, recipient, and nisin-producing transconjugants indicated that 68 kbp of DNA was transferred from the chromosome of the donor into the chromosome of the recipient in the conjugation process. The location of the transferred nisin structural gene *spaN* in the transconjugant HID500 was not stable, and cultures of strain HID500 were a mixture of different genotypes in which *spaN* was located at different positions in the chromosome on different *Sma*I fragments. *Apa*I, *Bgl*I, *Bss*HII, *Nci*I, *Sal*I, and *Sma*I digests of genomic DNA were used to map the location of *spaN* in a donor (DL11) and a nisin-producing transconjugant (HID504).

Some strains of *Lactococcus lactis* subsp. *lactis* have been known for many years to produce the bacteriocin nisin, a 34-residue polypeptide that inhibits the growth of a wide range of gram-positive bacteria including *Clostridium botulinum* (29) and *Listeria monocytogenes* (2). This property, along with its heat stability at acid pH and its susceptibility to degradation by the digestive enzyme α-chymotrypsin, has led to its extensive use as a food preservative, particularly in canned foods and dairy products (13).

Nisin belongs to a group of bacteriocins termed lantibiotics (25), which also includes the bacteriocins subtilin (11), epidermin (25), cinnamycin (10), duramycin (10), and gallidermin (15). These lantibiotics have similar sizes and structures and are characterized by the presence of the unusual amino acids lanthionine, β-methyllanthionine, dehydroalanine, and dehydrobutyrine.

The gene that encodes nisin precursor peptide (*spaN*) has been cloned, and its nucleotide sequence has been determined (4, 5, 14). The resulting data suggest that nisin is synthesized as a prepropeptide, containing a 23-amino-acid leader peptide and containing serine, threonine, or cysteine residues in positions which are modified posttranslationally to form lanthionine, β-methyllanthionine, dehydroalanine, and dehydrobutyrine (4, 5, 14). The molecular events resulting in cleavage of the leader peptide, posttranslational modifications of the nisin precursor peptide, and transport through the cell membrane are yet to be elucidated.

Genes encoding nisin resistance and nisin production can be transferred by conjugation from strains of *L. lactis* subsp. *lactis* into nisin-sensitive strains of *L. lactis* subsp. *lactis* (8, 9, 26), *L. lactis* subsp. *lactis* bv. diacetylactis (9), *Leuconostoc dextranicum* (33), and *L. lactis* subsp. *cremoris* (3, 26). Nisin-producing transconjugants also acquire both the ability to ferment sucrose (5, 8, 9, 26) and resistance to phage infection (9, 18), suggesting that the genes for sucrose fermentation and resistance to phage infection are transferred with the nisin genes. The observation by Dodd et al. (5) of an insertion sequence element (IS*904*) close to, or at the terminus of, the segment of DNA acquired by nisin-producing transconjugants raised the possibility of the involvement of this element in the conjugal transfer. Since this manuscript was submitted, Horn et al. (12) have shown that nisin genes are transferred by a conjugative transposon, Tn*5301*, which contains IS*904*. However, transposition of Tn*5301* did not appear to be dependent on IS*904*.

The location of the genes encoding nisin production and nisin resistance is unclear. Tsai and Sandine (33) detected an additional plasmid of 17.5 MDa in a nisin-producing transconjugant of *Leuconostoc dextranicum*, and Kaletta and Entian (14) isolated the nisin structural gene from plasmid DNA prepared from *L. lactis* subsp. *lactis* 6F3. By contrast, Dodd et al. (5), Steen et al. (27), and Horn et al. (12) presented evidence that *spaN* is located on the chromosome of nisin-producing strains of *L. lactis* subsp. *lactis*. In this paper, we describe the conjugal transfer of genes encoding nisin production and resistance between strains of lactococci and demonstrate that these genes are located in the chromosome of both the donor and the nisin-producing transconjugants. In addition, we found that the conjugal transfer of the nisin structural gene was associated with the transfer of a 68-kbp DNA fragment from the donor to the recipient. Sucrose-fermenting ability and resistance to infection by an isometric headed phage were also transferred in the same conjugation process.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacteria used in this study are listed in Table 1. Lactococci were grown in M17 medium (30) containing 0.5% (wt/vol) glucose (M17G). When required, erythromycin, streptomycin, fusidic acid, and nisin were used at concentrations of 10, 400, and 40 μg/ml and 500 IU/ml, respectively. Plasmid DNA was pre-

* Corresponding author.

TABLE 1. Bacterial strains and phages used in this study

Strain or phage	Relevant characteristics[a]	Source
Strains		
L. lactis subsp. *lactis*		
HID113	Sm^r Fus^r Nis^s Suc^- (Sm^r Fus^r mutant of LM0230)	21
LM0230(pMU1328)	Em^r Nis^s Suc^- (pMU1328 confers Em^r)	19
DL11	Nis^r Nis^P Suc^+ Rbs^+ (Prt^- derivative of ATCC 11454)	16, 18
HID500	Sm^r Fus^r Nis^r Nis^P Suc^+ Rbs^+ (transconjugant obtained by mating DL11 with HID113)	This study
HID501–HID505	Em^r Nis^r Nis^P Suc^+ Rbs^+ (transconjugants obtained by mating HID500 with LM0230(pMU1328)	This study
Micrococcus flavus NCIB 8166	Nis^s	32
Phages		
c2	Prolate headed bacteriophage infecting HID113 and LM0230	20
sk1	Isometric headed bacteriophage infecting HID113 and LM0230	20

[a] Abbreviations: Sm^r, streptomycin resistant; Fus^r, fusidic acid resistant; Em^r, erythromycin resistant; Nis^r, nisin resistant; Nis^P, nisin producing; Nis^s, nisin sensitive; Suc^+, sucrose fermenting; Rbs^+, reduced bacteriophage sensitivity.

pared by the method of Anderson and McKay (1) and purified by cesium chloride-ethidium bromide gradient centrifugation.

Conjugations. Overnight cultures of donor and recipient cells were inoculated (1:100) into fresh M17G and grown at 30°C to an A_{600} of 0.5. The cells were harvested by centrifugation, washed, and resuspended in the same volume of M17G. Equal volumes (1.0 ml) of the donor and recipient cells were mixed, and the cells were collected on membrane filters (pore size, 0.45 μm; Millipore). The filters were placed on M17G agar plates, and after incubation at 30°C for 3 h, the cells were washed off the filters with 2 ml of M17G and plated on selection plates containing nisin.

Detection of nisin-producing strains. Nisin-resistant transconjugants were tested for their ability to produce nisin. The transconjugants were grown overnight in M17G, and the cells were removed by filtration (pore size, 0.45 μm; Millipore). A portion (10 μl) of the filtrate from each transconjugant to be tested was spotted onto an M17G agar plate supplemented with 1% (vol/vol) Tween 20. After drying, 3.0 ml of soft M17G agar (0.5%, wt/vol) supplemented with 1.0% (vol/vol) Tween 20 and containing 10^6 CFU of the nisin-sensitive indicator strain *Micrococcus flavus* NCIB 8166 per ml (32) was added to the plate. Following overnight growth at 30°C, the presence of nisin in the filtrate was indicated by a clear zone surrounding the filtrate spot.

Sucrose fermentation and phage sensitivity tests. Nisin-producing transconjugants were tested for the ability to ferment sucrose by growth on indicator agar plates (17) containing 0.5% (wt/vol) sucrose instead of lactose. The phage sensitivity assay was performed as described previously (21), with the prolate and isometric headed phages c2 and sk1 (20), respectively.

Preparation of genomic DNA in agarose blocks and digestion. Genomic DNA was prepared in situ in agarose blocks, and the DNA in the blocks was prepared for digestion by restriction endonucleases as described by Tanskanen et al. (28). Restriction digests were carried out by using the temperatures and buffer conditions recommended by the enzyme manufacturer.

PFGE. DNA fragments were resolved by pulsed-field gel electrophoresis (PFGE) (35) through 1% (wt/vol) agarose gels in a buffer consisting of 45 mM Tris, 45 mM boric acid (pH 8.0), and 1 mM sodium EDTA, for 20 h at 200 V at 15°C in a Bio-Rad CHEF DRII electrophoresis cell (28). Gels were stained with ethidium bromide (1 mg/liter of water) to detect DNA. Fragment sizes were determined from their mobilities by using λ phage DNA concatemers (Pharmacia) and *Hin*dIII digests of λ DNA as molecular size standards.

Probe construction, radiolabeling and DNA hybridization. The DNA probe used to detect *spaN* was constructed by using the polymerase chain reaction. The oligonucleotide primers AAGGAGGCACTCAAAATGAGTAC (oligo I), corresponding to a region overlapping the ribosome-binding site preceding *spaN* (4), and TTATTTGCTTACGTGAAT ACTAC (oligo II), which is complementary to a region including the termination codon of *spaN* (4), were synthesized on an Applied Biosystems 381A DNA Synthesizer. The polymerase chain reaction amplification was carried out as recommended for the Perkin-Elmer Cetus GeneAmp DNA Amplification Reagent Kit (part no. N801-0043), with a concentration of 1 μM for each primer and 7.5 ng of DL11 genomic DNA as the template. A Hybaid intelligent heating block was run for 25 cycles of 94°C for 1 min, 37°C for 2 min, and 72°C for 2 min. The polymerase chain reaction product was separated by agarose gel electrophoresis, and the DNA fragment of the predicted size (ca. 200 bp) was recovered by electroelution onto NA45 paper (Schleicher & Schuell). The fragment was eluted from the paper with 1 M sodium chloride–50 mM arginine at 70°C for 2 h. ^{32}P-labeled probes were prepared by random priming (7) or by end labeling oligo I (described above) (24). Southern blots of PFGE agarose gels were prepared on nylon membranes (Du Pont or Gelman) by using an LKB VacuGene apparatus (Pharmacia) and 0.4 M NaOH as the eluant (23).

RESULTS

Mapping of *spaN* within donor strain DL11. We have previously constructed a physical map of the *L. lactis* subsp. *lactis* DL11 chromosome for the restriction enzymes *Sma*I and *Not*I (34). Hybridization experiments on PFGE separations of digests of total genomic DNA from strain DL11 (Fig. 1) showed that *spaN* is located on an 810-kbp *Sma*I fragment (*Sm*A) and a 600-kbp *Not*I fragment (*Nt*B). Since both of these fragments are chromosomal in origin, *spaN* must be located on the *L. lactis* subsp. *lactis* DL11 chromosome, specifically between the *Not*I and *Sma*I sites at map coordinates 1.98 and 2.50, respectively (34). The precise location of *spaN* with respect to the *Sma*I site was determined by

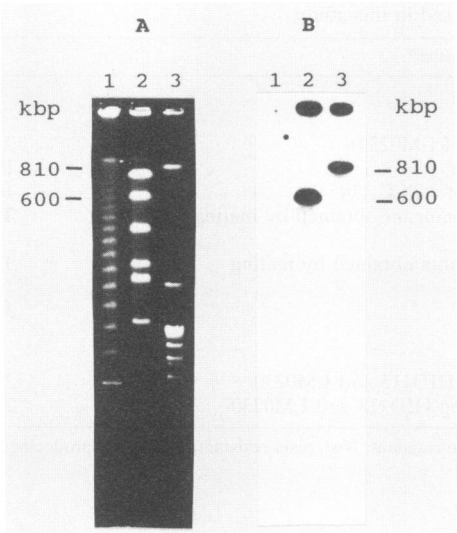

FIG. 1. PFGE of restriction endonuclease digests of *L. lactis* subsp. *lactis* DL11 genomic DNA. (A) Ethidium bromide-stained gel; (B) Southern blot of the gel shown in panel A hybridized with a ^{32}P-labeled *spaN* probe. Lanes: 1, phage λ DNA concatemers; 2, DL11 DNA digested with *Not*I; 3, DL11 DNA digested with *Sma*I.

additional hybridizations of the probe with PFGE separations of *Apa*I, *Bgl*I, *Bss*HII, *Nci*I, and *Sal*I digests of total genomic DNA (Fig. 2A). Since there is an *Nci*I site within *spaN* (4), it was also possible to determine the orientation of *spaN* on the chromosome (Fig. 2A) by using oligo I to probe a Southern blot of *Nci*I, *Nci*I-*Bgl*I, and *Nci*I-*Sal*I digests of DL11 DNA. The *spaN* probe did not hybridize with DL11 plasmid DNA (Fig. 3B, lane 10).

Conjugal transfer of *spaN*. *L. lactis* subsp. *lactis* DL11 was used as the donor in a conjugation experiment with *L. lactis* subsp. *lactis* HID113 (Table 1) as the recipient. One nisin-resistant (Nis^r), Sm^r, Fus^r transconjugant (HID500) was obtained in this experiment (conjugation frequency of 10^{-7} per donor). In addition to being nisin resistant, this transconjugant produced nisin, was able to ferment sucrose, and exhibited reduced sensitivity to infection by the small isometric headed phage sk1 compared with that of the recipient strain HID113. The sensitivity of the transconjugant to the prolate headed phage c2 was the same as that of the recipient strain HID113. Samples of high-molecular-weight genomic DNA isolated from the transconjugant, the donor, and the recipient were digested with *Sma*I, and the resulting fragments were resolved by PFGE (Fig. 3A, lanes 1 to 3). Strain HID500 contained four *Sma*I fragments of 427, 398, 252, and 155 kbp, which were not present in the recipient strain HID113. The *spaN* probe hybridized to three of the new *Sma*I fragments in strain HID500 (the fragments of 398, 252, and 155 kbp) (Fig. 3B, lane 2). The *spaN* probe hybridized most intensely with the 252-kbp fragment.

It has been shown previously (28), and was confirmed in this study, that the genome of LM0230 (and its derivative HID113) contains two 184-kbp *Sma*I fragments. By contrast, densitometric analysis of the electropherogram shown in Fig. 3A revealed that the molar ratio of the 184-kbp fragment(s) in HID500 was approximately 1.3, suggesting that strain HID500 contained submolar amounts of one of the two 184-kbp *Sma*I fragments present as a doublet in strain HID113 (Fig. 3A, lanes 1 and 2). This was confirmed by hybridization studies (see below and Fig. 6). Densitometric analyses also showed that the 427-, 398-, and 155-kbp *Sma*I fragments were all present in submolar amounts. This raised the possibility that the original transconjugant was genetically unstable and had given rise to a genetically heterogeneous population of cells during the course of its isolation and subsequent growth for genomic DNA preparation.

To examine the genetic stability of HID500, a sample was plated out, 10 single colonies were isolated at random, and genomic DNA was prepared from them. The PFGE *Sma*I digestion patterns of seven of these new isolates were identical (Fig. 4A, lanes 3, 5, and 7 to 11). By comparison with strain HID113 (Fig. 3, lane 1), they each contained an additional band (of 252 kbp) and lacked one of the 184-kbp fragments. The *spaN* probe hybridized only to the 252-kbp fragment in these isolates (Fig. 4B). The DNA isolated from the other three colonies contained more than one additional *Sma*I fragment compared with strain HID113 and contained a number of *Sma*I fragments that hybridized with *spaN* (Fig.

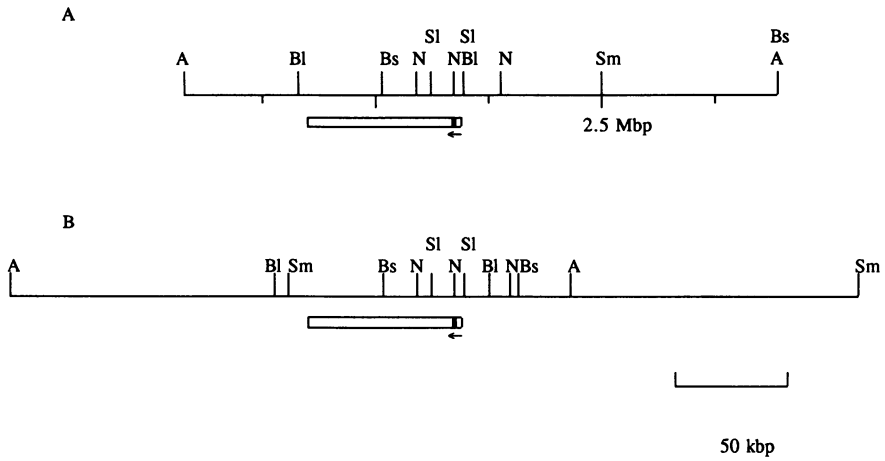

FIG. 2. Restriction map of the *spaN* region of the chromosomes of *L. lactis* subsp. *lactis* DL11 (A) and HID504 (B). Restriction enzymes: A, *Apa*I; Bl, *Bgl*I; Bs, *Bss*HII; N, *Nci*I; Sl, *Sal*I; Sm, *Sma*I. The boxes under the maps represent the 68-kbp of DNA transferred in conjugation experiments. The solid portion of these boxes locates *spaN*. The arrows under the boxes show the direction of transcription of *spaN*. The number under the DL11 map refers to the position on the physical map of this organism (34).

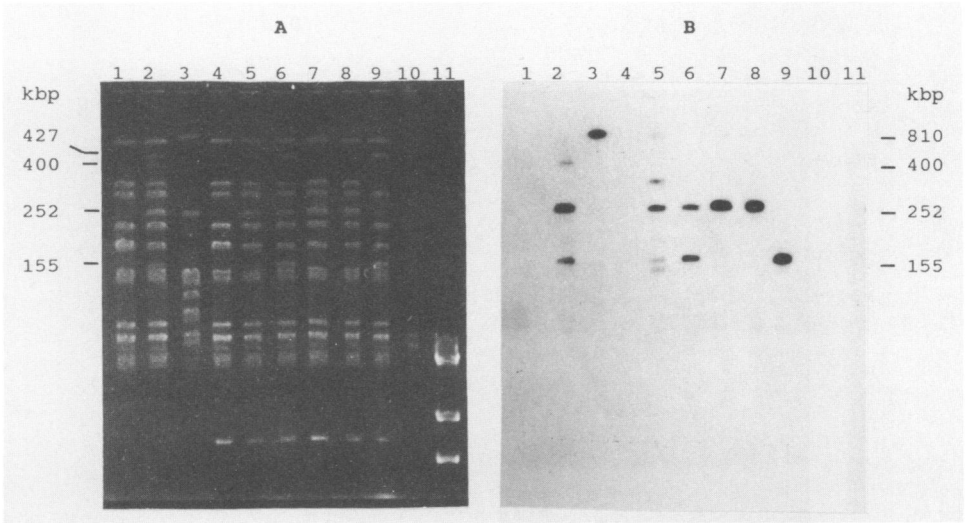

FIG. 3. PFGE of *Sma*I restriction digests of genomic DNA from donor, recipient, and transconjugants of *L. lactis* subsp. *lactis*. (A) Ethidium bromide-stained gel; (B) Southern blot of the gel shown in panel A hybridized with a ^{32}P-labeled *spaN* probe. Lanes: 1 to 10, *Sma*I digests of genomic DNA from HID113 (lane 1), HID500 (lane 2), DL11 (lane 3), LM0230(pMU1328) (lane 4), HID501 (lane 5), HID502 (lane 6), HID503 (lane 7), HID504 (lane 8), HID505 (lane 9), and DL11 plasmid DNA (lane 10); lane 11, phage λ digested with *Hin*dIII.

4, lanes 4, 6, and 12). The 252-kbp *Sma*I fragment was present in submolar amounts in the DNA from one of these colonies (Fig. 4A, lane 6) and was not visible in the restriction pattern of DNA from another (Fig. 4A, lane 12). None of the colonies yielded *Sma*I restriction patterns, or hybridization patterns with *spaN*, which were identical with that of HID500. Indeed, samples of DNA isolated from new cultures of strain HID500 gave a different hybridization pattern from that obtained originally (Fig. 3A, lane 2). We conclude from these results that the location of *spaN* in the original transconjugant was not stable and that the cultures of strain HID500 were a mixture of different genotypes in which *spaN* was located at different positions in the chromosome on different *Sma*I fragments.

Secondary conjugation. The ability of strain HID500 to act as a donor of nisin production was tested in a conjugation experiment with *L. lactis* subsp. *lactis* LM0230(pMU1328) (Table 1) as the recipient. Five Nis^r Em^r transconjugants (HID501 to HID505) obtained from this mating were selected for further study. All the transconjugants produced nisin, were able to ferment sucrose, and exhibited reduced sensitivity to infection by phage sk1 compared with that of the recipient strain LM0230(pMU1328), whereas susceptibility to c2 phage remained unchanged.

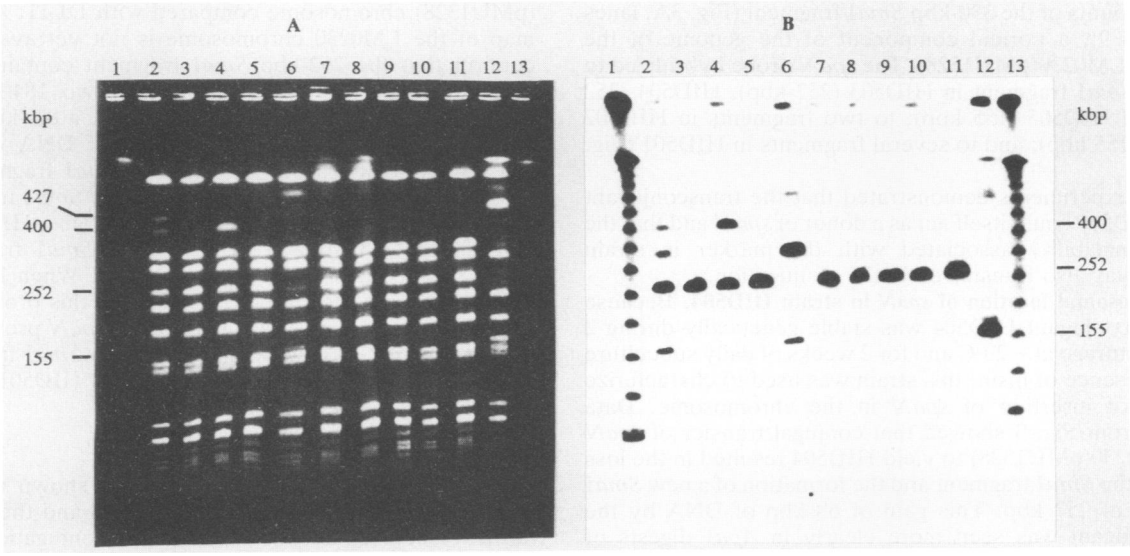

FIG. 4. PFGE of *Sma*I restriction digests of genomic DNA from individual colonies isolated from HID500. (A) Ethidium bromide-stained gel; (B) Southern blot of the gel shown in panel A hybridized with ^{32}P-labeled *spaN* and phage λ DNA as the probes. Lanes: 1, 13, phage λ DNA concatemers; 2, *Sma*I digest of original transconjugant HID500; 3 to 12, *Sma*I digests of DNA from 10 single colonies isolated from HID500.

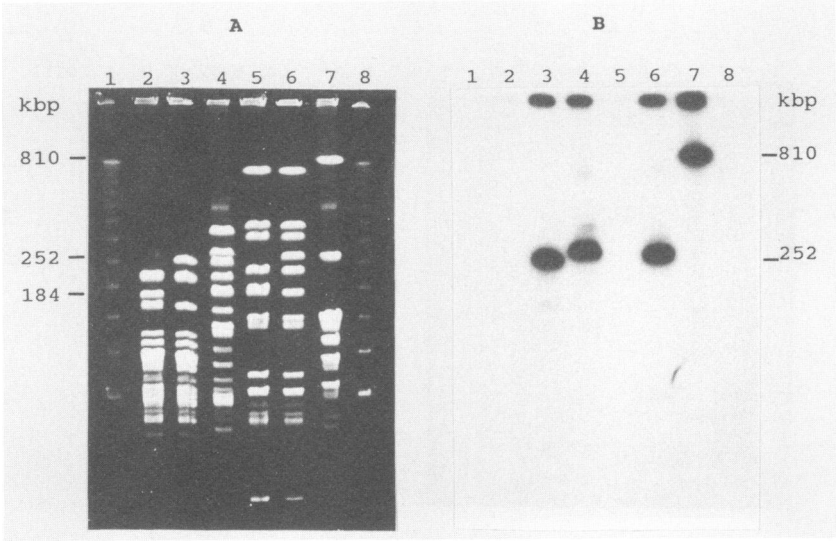

FIG. 5. PFGE of restriction endonuclease digests of *L. lactis* subsp. *lactis* LM0230(pMU1328), DL11, and HID504 genomic DNA. (A) Ethidium bromide-stained gel; (B) Southern blot of the gel shown in panel A hybridized with ^{32}P-labeled *spaN* DNA as the probe. Lanes: 1 and 8, phage λ DNA concatemers; 2 to 7, *Apa*I digests of LM0230(pMU1328) (lane 2), HID504 (lane 3), and DL11 (lane 4) and *Sma*I digests of LM0230(pMU1328) (lane 5), HID504 (lane 6), and DL11 (lane 7).

The *Sma*I digestion patterns of genomic DNA isolated from all of these transconjugants (Fig. 3A, lanes 5 to 9) contained one or more bands that were not present in the pattern of the recipient strain, LM0230(pMU1328) (Fig. 3A, lane 4). Thus, transconjugants HID503 and HID504 (Fig. 3A, lanes 7 and 8) contained one additional *Sma*I fragment (of 252 kbp); transconjugant HID505 (Fig. 3A, lane 9) contained two additional *Sma*I fragments (of 427 and 155 kbp), and transconjugants HID501 and HID502 (Fig. 3A, lanes 5 and 6) each contained three additional *Sma*I fragments (of 427, 252, and 155 kbp). All five transconjugants were missing one of the 184-kbp *Sma*I fragments present as a doublet in the recipient strain, whereas the three transconjugants that contained the 427-kbp *Sma*I fragment contained only submolar amounts of the 330-kbp *Sma*I fragment (Fig. 3A, lanes 5, 6, and 9), a normal component of the genome of the recipient LM0230(pMU1328). The *spaN* probe hybridized to a single *Sma*I fragment in HID503 (252 kbp), HID504 (252 kbp), and HID505 (155 kbp); to two fragments in HID502 (252 and 155 kbp); and to several fragments in HID501 (Fig. 3B).

These experiments demonstrated that the transconjugant strain HID500 could itself act as a donor of *spaN* and that the genetic instability associated with the marker in strain HID500 was also transferred in the conjugation.

Chromosomal location of *spaN* in strain HID504. Because the transconjugant HID504 was stable genetically during 2 years of storage at −20°C and for 2 weeks of daily subculture in the presence of nisin, this strain was used to characterize the site of insertion of *spaN* in the chromosome. Data derived from Fig. 3 showed that conjugal transfer of *spaN* into LM0230(pMU1328) to yield HID504 resulted in the loss of a 184-kbp *Sma*I fragment and the formation of a new *Sma*I fragment of 252 kbp. This gain of 68 kbp of DNA by the transconjugant was seen more clearly in *Apa*I digests of DNA from strains HID504 and LM0230(pMU1328) (Fig. 5). An *Apa*I fragment, coincidentally also of 184 kbp, present in the recipient (lane 2) was replaced in the transconjugant HID504 by a new fragment of 252 kbp (lane 3). This 252-kbp fragment was the only fragment that hybridized with the *spaN* probe. These data, together with those from the *Sma*I digests, indicate that the conjugal transfer of genes encoding nisin production and resistance was associated with the insertion of a 68-kbp DNA fragment containing *spaN* into the chromosome of the recipient. The location and orientation of *spaN* in the HID504 chromosome were mapped as described above for DL11 (Fig. 2B). The restriction map of the region surrounding *spaN* and extending for at least 32 kbp downstream was identical in both HID504 and the original donor strain DL11. However, the restriction map outside this region differed between the two strains (Fig. 2). This suggested that the 68-kbp DNA fragment containing *spaN* inserted into a different region of the LM0230 (pMU1328) chromosome compared with DL11. A physical map of the LM0230 chromosome is not yet available. To confirm that the 252-kbp *Sma*I fragment containing *spaN* was indeed derived from one of the two 184-kbp *Sma*I fragments present in the recipient strain, a 8.5-kbp *Bss*HII fragment located outside the 68 kbp of DNA containing *spaN* was isolated from the 252-kbp *Sma*I fragment. This *Bss*HII fragment hybridized to a 184-kbp *Sma*I fragment in both of the conjugation recipients [strains HID113 and LM0230(pMU1328)] and to a 145-kbp *Sma*I fragment in strain DL11, the original donor (Fig. 6). When hybridized with DNA from the six transconjugants, this probe hybridized to the same *Sma*I fragments as the *spaN* probe, except that it did not hybridize with the 398-kbp *Sma*I fragment in HID500 or the 145-kbp *Sma*I fragment in HID501 (Fig. 6).

DISCUSSION

Previous workers (8, 9, 26, 33) have shown that genes encoding nisin resistance and production and the ability to ferment sucrose are cotransferred in conjugation experiments. The location of the nisin genes has been contentious. Early conjugation experiments (26, 33) suggested a plasmid location of these genes, and Kaletta and Entian (14) cloned the gene encoding the nisin precursor peptide from plasmid

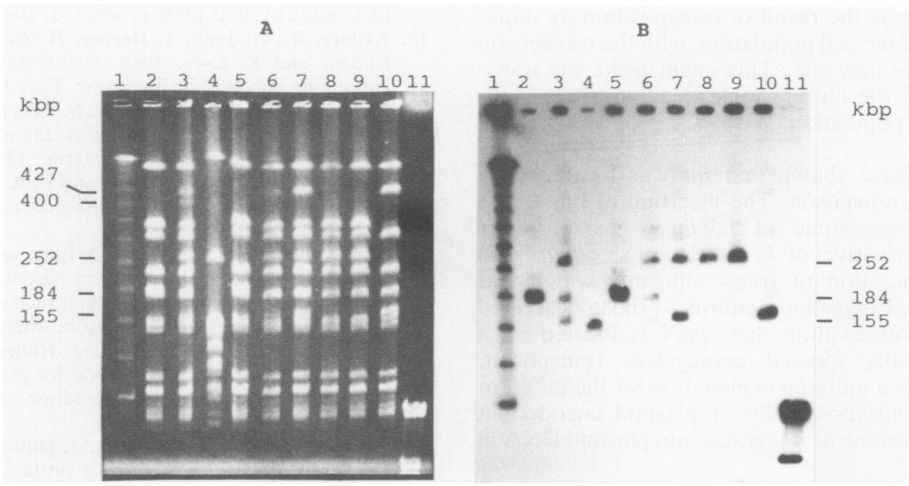

FIG. 6. PFGE of *Sma*I restriction digests of genomic DNA from donors, recipients, and transconjugants of *L. lactis* subsp. *lactis*. (A) Ethidium bromide-stained gel; (B) Southern blot of the gel shown in panel A hybridized with the ^{32}P-labeled 8.5-kbp *Bss*HII fragment (described in the text) and λ DNA probes. Lanes: 1, phage λ DNA concatemers; 2 to 10, *Sma*I digests of genomic DNA from HID113 (lane 2), HID500 (lane 3), DL11 (lane 4), LM0230(pMU1328) (lane 5), HID501 (lane 6), HID502 (lane 7), HID503 (lane 8), HID504 (lane 9), and HID505 (lane 10); 11, phage λ DNA digested with *Hin*dIII.

DNA in *L. lactis* subsp. *lactis* 6F3. By contrast, Dodd et al. (5) concluded that the nisin precursor gene was chromosomally located in *L. lactis* subsp. *lactis* FI5876 on the basis of hybridization studies with various gene probes. Steen et al. (27) concluded that the nisin precursor gene is chromosomally located in *L. lactis* subsp. *lactis* ATCC 11454 since gene probes hybridized with DNA fragments which were too large to be derived from plasmids.

We have used PFGE analysis of digests of *L. lactis* subsp. *lactis* DL11 to show unequivocally that *spaN* is located on the chromosome of this strain. *spaN* hybridized to an 810-kbp *Sma*I fragment and a 600-kbp *Not*I fragment which we have previously located on the physical map of the DL11 chromosome (34). We were thus able to determine the location of *spaN* with respect to the *Sma*I site at position 2.5 Mbp on this map. The DL11 strain used in this study is a spontaneous Prt$^-$ derivative of the *L. lactis* subsp. *lactis* ATCC 11454 studied by Steen et al. (27). These authors found that *spaN* hybridized to a 625-kbp *Not*I fragment and to a 140-kbp *Sma*I fragment. The reason for the discrepancy in the size of the *Sma*I fragment containing *spaN* is not known.

Our work shows that the conjugal transfer of *spaN* results in the insertion of a 68-kbp fragment containing *spaN* into the chromosome of the recipient strain. In addition to *spaN*, the transferred fragment presumably contains genes encoding nisin immunity or resistance, nisin-modifying enzymes, reduced bacteriophage sensitivity, and sucrose-utilizing ability. Donkersloot and Thompson (6) have reported that a chromosomal deletion in the nisin-producing strain *L. lactis* subsp. *lactis* K1 resulted in the loss of nisin resistance, nisin production, sucrose-fermenting ability, and N^5-(carboxyethyl)ornithine synthase. However, sucrose- and nisin-deficient mutants of ATCC 11454 (the parent strain of DL11) are still capable of producing N^5-(carboxyethyl)ornithine, suggesting that the gene encoding N^5-(carboxyethyl)ornithine synthase (*ceo*) is not part of the transposon in this strain (31). Thompson et al. have shown that the structural genes encoding enzyme II^{scr} (*scrA*) and sucrose-6-phosphate hydrolase (*scrB*) are closely linked with *ceo* in strain K1, but not in ATCC 11454 (31). The insertion sequence IS*904* has also been closely linked to *spaN* (5, 12, 22).

Two types of stable transformants containing a single location of *spaN* were observed in this study. The predominant transconjugant type (HID503 and HID504) contained a 252-kbp *Sma*I fragment which replaced the 184-kbp *Sma*I fragment present in the recipient. *spaN* probes hybridized to the 252-kbp fragment. An 8.5-kbp *Bss*HII fragment isolated from the 252-kbp *Sma*I fragment, but outside the transferred 68-kbp region containing *spaN*, hybridized with one of the 184-kbp *Sma*I fragments present in the recipient strain. We conclude, therefore, that this class of transconjugant is the result of the insertion of a 68-kbp fragment from the donor strain into one of the 184-kbp *Sma*I fragments of the recipient.

The second type of stable transformant observed in this study included transconjugants of the HID505 type. These transconjugants contained *Sma*I fragments of 427 and 155 kbp which were not present in the recipient strain and lacked the 330-kbp *Sma*I fragment present in the recipient. Both the *spaN* probe and the 8.5-kbp *Bss*HII fragment hybridized to the 155-kbp *Sma*I fragment. This class of transconjugant may be the result of insertion of the 68-kbp transferred fragment into the 184-kbp *Sma*I fragment of the recipient, followed by a recombination event between the newly formed 252-kbp *Sma*I fragment and the 330-kbp fragment present in the recipient strain.

In addition to the stable type of transconjugants described above, some transconjugants contained a 398-kbp *Sma*I fragment containing *spaN*. This could be the result of insertion of the 68-kbp segment into the 330-kbp *Sma*I fragment present in the recipient strain and present in submolar amounts in some of the transconjugants.

The reason for obtaining multiple *Sma*I fragments containing *spaN* in some of the transconjugants is not known. Our results do not enable us to determine whether cultures of these transconjugants include individual cells carrying multiple copies of *spaN* in various *Sma*I fragments of the genome, or a mixed population of cells, all containing a single copy of *spaN* in different *Sma*I fragments. Multiple

copies of *spaN* may be the result of transposition by duplication in a portion of the cell population, with the transposon being unstable at the new site. This might make the transposon move around the chromosome, leading to a genetically heterogeneous population. Work is in progress to study this phenomenon.

Horn et al. (12) have shown that *spaN* is located on a 70-kbp conjugative transposon. The insertion of this transposon into the chromosome of *L. lactis* subsp. *lactis* MG1614 (also a derivative of *L. lactis* subsp. *lactis* C2) resulted in the generation of transconjugants which had similar genomic *Sma*I digestion patterns to those described in this work. The observation that *spaN* is located on a 70-kbp chromosomally located conjugative transposon, Tn*5301*, may provide a unifying explanation for the different location of *spaN* (chromosomally or plasmid encoded) if Tn*5301* acts as an episome or integrates into plasmid DNA in some strains.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the Australian Research Council. T.G. is the recipient of an award from the Commonwealth Scholarship and Fellowship Plan.

We thank Phil Arnold for synthesizing the oligonucleotides and Malcolm Hickey and John Coventry for helpful discussions.

REFERENCES

1. **Anderson, D. G., and L. L. McKay.** 1983. A simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Environ. Microbiol. **46:**549–552.
2. **Benkerroum, N., and W. E. Sandine.** 1988. Inhibitory action of nisin against *Listeria monocytogenes* J. Dairy Sci. **71:**3237–3245.
3. **Broadbent, J. R., and J. K. Kondo.** 1991. Genetic construction of nisin producing *Lactococcus lactis* subsp. *cremoris* and analysis of a rapid method for conjugation. Appl. Environ. Microbiol. **57:**517–524.
4. **Buchman, G. W., S. Banerjee, and J. N. Hansen.** 1988. Structure, expression, and evolution of a gene encoding the precursor of nisin, a small protein antibiotic. J. Biol. Chem. **263:**16260–16266.
5. **Dodd, H. M., N. Horn, and M. J. Gasson.** 1990. Analysis of the genetic determinant for production of the peptide antibiotic nisin. J. Gen. Microbiol. **136:**555–566.
6. **Donkersloot, J. A., and J. Thompson.** 1990. Simultaneous loss of N5 (carboxyethyl) ornithine synthase, nisin production, and sucrose-fermenting ability. J. Bacteriol. **172:**4122–4126.
7. **Feinberg, A. P., and B. Vogelstein.** 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137:**266–267.
8. **Gasson, M. J.** 1984. Transfer of sucrose fermenting ability, nisin resistance and nisin production into *Streptococcus lactis* 712. FEMS Microbiol. Lett. **21:**7–10.
9. **Gonzalez, C. F., and B. S. Kunka.** 1985. Transfer of sucrose-fermenting ability and nisin production phenotype among lactic streptococci. Appl. Environ. Microbiol. **49:**627–633.
10. **Gross, E.** 1977. α,β-Unsaturated and related amino acids in peptides and proteins. Adv. Exp. Med. Biol. **86b:**131–153.
11. **Gross, E., and H. H. Kiltz.** 1973. The number and nature of α,β-unsaturated amino acids in subtilin. Biochem. Biophys. Res. Commun. **50:**559–565.
12. **Horn, N., S. Swindell, H. Dodd, and M. Gasson.** 1991. Nisin biosynthesis genes are encoded by a novel conjugative transposon. Mol. Gen. Genet. **228:**129–135.
13. **Hurst, A.** 1981. Nisin. Adv. Appl. Microbiol. **27:**85–123.
14. **Kaletta, C., and K.-D. Entian.** 1989. Nisin, a peptide antibiotic: cloning and sequencing of the *nisA* gene and posttranslational processing of its peptide product. J. Bacteriol. **171:**1597–1601.
15. **Kellner, R., G. Jung, T. Horner, H. Zahner, N. Schnell, K.-D. Entian, and F. Gotz.** 1988. Gallidermin, a new lanthionine containing polypeptide antibiotic. Eur. J. Biochem. **177:**53–59.
16. **Le Blanc, D. J., V. L. Crow, L. N. Lee, and C. F. Garon.** 1979. Influence of the lactose plasmid on the metabolism of galactose by *Streptococcus lactis*. J. Bacteriol. **137:**878–884.
17. **McKay, L. L., K. A. Baldwin, and E. A. Zottola.** 1972. Loss of lactose metabolism in lactic streptococci. Appl. Environ. Microbiol. **23:**1090–1096.
18. **Murphy, M. C., J. L. Steele, C. Daly, and L. L. McKay.** 1988. Concomitant conjugal transfer of reduced bacteriophage sensitivity mechanisms with lactose and sucrose-fermenting ability in lactic streptococci. Appl. Environ. Microbiol. **54:**1951–1956.
19. **Powell, I. B., M. G. Achen, A. J. Hillier, and B. E. Davidson.** 1988. A simple and rapid method for genetic transformation of lactic streptococci by electroporation. Appl. Environ. Microbiol. **54:**655–660.
20. **Powell, I. B., P. M. Arnold, A. J. Hillier, and B. E. Davidson.** 1989. Molecular comparison of prolate and isometric-headed bacteriophages of lactococci. Can. J. Microbiol. **35:**860–866.
21. **Powell, I. B., A. C. Ward, A. J. Hillier, and B. E. Davidson.** 1990. Simultaneous conjugal transfer in *Lactococcus* of genes involved in bacteriocin production and reduced susceptibility to bacteriophages. FEMS Microbiol. Lett. **72:**209–214.
22. **Rauch, P. J. G., M. M. Beerthuyzen, and W. M. de Vos.** 1990. Nucleotide sequence of IS904 from *Lactococcus lactis* subsp. *lactis* strain NIZO R5. Nucleic Acids Res. **18:**4253.
23. **Reed, K. C., and D. A. Mann.** 1985. Rapid transfer of DNA from agarose gels to nylon membrane. Nucleic Acids Res. **13:**7207–7221.
24. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Schnell, N., K. D. Entian, U. Schneider, F. Gotz, H. Zahner, R. Kellner, and G. Jung.** 1988. Peptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings. Nature (London) **333:**276–278.
26. **Steele, J. L., and L. L. McKay.** 1986. Partial characterization of the genetic basis for sucrose metabolism and nisin production in *Streptococcus lactis*. Appl. Environ. Microbiol. **51:**57–64.
27. **Steen, M. T., Y. J. Chung, and N. Hansen.** 1991. Characterization of the nisin gene as part of a polycistronic operon in the chromosome of *Lactococcus lactis* ATCC 11454. Appl. Environ. Microbiol. **57:**1181–1188.
28. **Tanskanen, E. I., D. L. Tulloch, A. J. Hillier, and B. E. Davidson.** 1990. Pulsed-field gel electrophoresis of *Sma*I digests of lactococcal genomic DNA, a novel method of strain identification. Appl. Environ. Microbiol. **56:**3105–3111.
29. **Taylor, S. M., and E. B. Somers.** 1985. Evaluation of the antibotulinal effectiveness of nisin in bacon. J. Food Prot. **48:**949–952.
30. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29:**807–813.
31. **Thompson, J., N. Y. Nguyen, D. L. Sacket, and J. A. Donkersloot.** 1991. Transposon-encoded sucrose metabolism in *Lactococcus lactis*. J. Biol. Chem. **226:**14573–14579.
32. **Tramer, J., and G. G. Fowler.** 1964. Estimation of nisin in foods. J. Sci. Food Agric. **15:**522–528.
33. **Tsai, H. J., and W. E. Sandine.** 1987. Conjugal transfer of nisin plasmid genes from *Streptococcus lactis* 7962 to *Leuconostoc dextranicum* 181. Appl. Environ. Microbiol. **53:**352–357.
34. **Tulloch, D. L., L. R. Finch, A. J. Hillier, and B. E. Davidson.** 1991. Physical map of the chromosome of *L. lactis* subsp. *lactis* DL11 and the localization of six putative rRNA operons. J. Bacteriol. **173:**2768–2775.
35. **Volrath, D., and R. W. Davis.** 1987. Resolution of DNA molecules greater than 5 megabases by contour-clamped homogeneous electric fields. Nucleic Acids Res. **15:**7865–7876.