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Molecular and Genetic Characterization of a Novel Nisin Variant Produced by *Streptococcus uberis*

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Streptococcus uberis **is one of the principal causative agents of bovine mastitis. In this study, we report that *S. uberis* strain 42 produces a lantibiotic, nisin U, which is 78% identical (82% similar) to nisin A from *Lactococcus lactis*. The 15.6-kb nisin U locus comprises 11 open reading frames, similar in putative functionality but differing in arrangement from that of the nisin A biosynthetic cluster. The nisin U producer strain exhibits specific resistance (immunity) to nisin U and cross-resistance to nisin A, a finding consistent with the 55% sequence similarity of their respective immunity peptides. Homologues of the nisin U structural gene were identified in several additional *S. uberis* strains, and in each case cross-protective immunity was expressed to nisin A and to the other producers of nisin U and its variants. To our knowledge, this is the first report both of characterization of a bacteriocin by *S. uberis*, as well as of a member of the nisin family of peptides in a species other than *L. lactis*.**

The lactic acid bacterium *Streptococcus uberis*, in nature primarily found on the lips and skin of cows, in raw milk, and on udder tissue (11), is also a major cause of bovine mastitis (4). Due to the ubiquity of this bacterium in the environment of the dairy cow, teat-end contamination poses a constant threat of infection, and the current level of disease caused by *S. uberis* remains a persistent problem impacting both on the economic production of milk and on the welfare of the dairy cow (21).

Many lactic acid bacteria produce broad-spectrum proteinaceous antimicrobials called bacteriocins, some of which could provide valuable alternatives to traditional therapeutic antibiotics for the treatment of infectious diseases (30). Two such bacteriocins, nisin and lacticin 3147, which are produced by strains of *Lactococcus lactis* are potential candidates for mastitis control (5, 30). Nisin is the active ingredient in two commercial products: Consept (Applied Microbiology, Inc., New York, NY) and Wipe-Out (ImmuCell, Portland, OR). Lacticin 3147 has also been evaluated as a teat-seal formulation for the prevention of mastitis during the "dry" period in which the cow is not lactating (30).

Nisin and lacticin 3147 both belong to the lantibiotic class of bacteriocins (10, 34). The lantibiotics are ribosomally synthesized, low-molecular-weight, heat-stable peptides characterized by their content of posttranslationally modified amino acids, including lanthionine and/or β-methyl-lanthionine (22, 26, 31). Nisin is the most intensively studied lantibiotic (20, 35, 37). Nisin Z (25) and nisin Q (45) are two natural variants of the original nisin A, differing in their propeptide components from nisin A by one and four amino acids, respectively. Lantibiotic loci typically comprise a structural gene (*lanA*) and other genes that encode proteins responsible for posttranslational modification of the prepeptide (*lanB* and *lanC*, or *lanM*), proteolytic processing (*lanP*), transport (*lanT*), producer self-protection (*lanI* and *lanEFG*), and regulation of biosynthesis (*lanR*, *lanK*, and *lanQ*) (10, 22). The lantibiotics described to date display considerable heterogeneity, both in their propeptide compositions and in the order and orientation of their gene clusters (36). Moreover, not all lantibiotic producer strains have the same complement of *lan* genes, this being a reflection of the considerable variety of posttranslational modifications introduced and in the mechanisms of processing, immunity and regulation of the different lantibiotics (3).

In this laboratory, a set of nine indicator bacteria is routinely used to detect and differentiate between the various bacteriocin-like inhibitory substances (BLIS) produced by streptococci. The patterns of inhibitory activity against these indicators, when expressed in code format are called BLIS production (P) types. Although it has been known for almost 20 years that *S. uberis* strains very commonly produce BLIS and that these are of a great variety of P types (6, 15, 18, 39), none of the inhibitory agents have yet been fully characterized. The strong activity of many of these BLIS against mastitis pathogens has prompted interest in their potential application to the control of udder infections. We report here the molecular characterization of nisin U, a novel nisin variant, by *Streptococcus uberis* strain 42.

MATERIALS AND METHODS

Bacterial strains, media, and culture conditions. The bacterial strains used in the present study are listed in Table 1. The nine standard indicator strains used for detection of BLIS activity have been previously described (38). Routine culture was on blood agar plus calcium carbonate (BACa) (Columbia Agar Base; Becton Dickinson [BD], Sparks, Md.; supplemented with 5% [vol/vol] whole human blood and 0.1% [wt/vol] $CaCO_3$) or in Todd-Hewitt broth (THB; BD) and at 37°C in 5% CO_2 in air for 18 h. All cloning experiments utilized the *Escherichia coli* DH10B host, which was grown in Luria broth (LB) plus 100 μg of ampicillin (Amp)/ml and on LB plus Amp agar (1% [wt/vol] tryptone [BD], 0.5% [wt/vol] yeast extract [BD], 1% [wt/vol] NaCl, 1.5% bacteriological agar [Scientific Supplies, Ltd., Auckland, New Zealand], 100 μg of Amp/ml).

Spectrum of inhibitory activity. The range of inhibitory activity of the BLIS-producing strains was detected by the deferred antagonism method, as described previously by Tagg and Bannister (38). The inhibitory activity of purified lan-

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TABLE 1. List of bacterial strains used in this study and their relevant characteristics

Species	Strain specification	Notes	Source or reference(s)
Streptococcus uberis	ATCC 27958	Also indicator I-4 in the set of standard indicators	38
	D535, D536, NY-41		39
	42, J4, J115, E		B. Jayarao
	B10a		B. Buddle
	RF1384, 71-612		Laboratory collection
L. lactis	ATCC 1404	Nisin A producer	Laboratory collection
	A5	Nisin Z producer	
	T-21	Also indicator I-6 in the set of standard indicators	38
S. pyogenes	FF22	Streptococcin A-FF22 producer, also indicator I-2 in the set of standard indicators	14, 38
	71-679	Indicator I-5	
	71-698	Indicator I-7	
	W-1	Indicator I-8	
S. anginosus	T-29	Indicator I-3	38
S. equisimilis	T-148	Indicator I-9	38
M. luteus	T-18	Indicator I-1	38

tibiotic preparations was determined by spot assays (43). Briefly, 20-μl aliquots of the preparation to be tested (and of doubling dilutions in sterile saline [0.85% [wt/vol] NaCl]) were applied to the surface of BACa medium and allowed to dry. The agar surface was then sterilized by exposure to chloroform vapor for 30 min and air dried for 30 min. The indicator organism (*Micrococcus luteus* T-18), was then applied onto the entire agar surface with a cotton swab. The titer, in arbitrary units (AU) per milliliter, is defined as the reciprocal of the highest dilution of the preparation for which a definite zone of inhibition is observed.

Extraction and purification of nisin U. One liter of Columbia broth (BD) was inoculated with a 1% (vol/vol) inoculum from an 18-h Columbia broth culture of *S. uberis* 42 and incubated for 18 h at 37°C in a 5% CO_2 in air atmosphere, after which the cells were recovered by centrifugation (15,300 × *g* for 15 min at 4°C). The inhibitory agent was extracted at 4°C for 18 h from the cell pellet with 100 ml of 50 mM HCl in saline. Sequential column purification utilized buffer A (10% [vol/vol] acetonitrile plus 0.1% [vol/vol] trifluoroacetic acid [TFA]) and buffer B (90% [vol/vol] acetonitrile plus 0.1% [vol/vol] TFA). An initial reversed-phase high-pressure liquid chromatography (RP-HPLC) step used a Brownlee Aquapore column (particle size, 7 μm; 300-Å pore; 4.6 by 40 mm [Alltech, Deerfield, IL]) and was developed at a flow rate of 1 ml/min using a gradient of 0 to 100% buffer B over 30 min. Active fractions were pooled, and acetonitrile evaporated using a Model 5301 vacuum concentrator (Eppendorf GmbH, Hamburg, Germany). A second RP-HPLC step was carried out using a 2-by-60-mm Nucleosil ODS-120 column (particle size, 3 μm; 120-Å pore [Grom HPLC & Analytik, Herrenberg, Germany]) at a flow rate of 0.4 ml/min, with a gradient of 0 to 60% buffer B over 30 min. Active fractions were pooled, the acetonitrile was removed by evaporation, and fractions were applied to a 2-by-250-mm Nucleosil ODS-120 column (particle size, 5 μm; 120-Å pore [Grom]) and further purified by RP-HPLC at a flow rate of 0.3 ml/min by using a gradient of 0 to 35% buffer B over 55 min.

MS and N-terminal amino acid sequence analysis. Mass spectrometry (MS) was conducted by electrospray ionization (ESI) using a LCQ Deca mass spectrometer (ThermoFinnigan, Hemel Hempstead, United Kingdom). Positive-mode ESI-MS data were accumulated by introduction of peptide samples (dissolved in 30% aqueous acetonitrile containing 0.1% TFA) via a syringe pump into the ESI interface or directly injected into solvent flow. Mass spectra were analyzed by using the manufacturer's software. The N-terminal amino acid sequence of peptides was obtained by using a Model 492 Procise HT-pulsed gas-liquid microsequencer (Applied Biosystems, Foster City, CA). Samples for automated Edman degradation were applied to a TFA-treated glass fiber support (13). Prior to N-terminal sequencing, the peptide was derivatized by using the method of Meyer et al. (23) as modified by Walk et al. (42). Mass analyses and N-terminal sequencing were carried out at the Protein Microchemistry Facility (Department of Biochemistry, University of Otago).

DNA extraction and primer walking. The general molecular cloning techniques and DNA detection assays were essentially as described by Sambrook et al. (33). DNA was extracted from streptococci by using the DNeasy Tissue Kit (QIAGEN, Hilden, Germany) as per the supplier's recommendations or by a LiCl extraction method. The latter is briefly as follows. Cells from a 3-ml 18-h culture (in THB) were harvested (4,500 × *g* for 3 min), resuspended in 300 μl of TE buffer containing 7 mg of lysozyme/ml and 30 U of mutanolysin/ml, and incubated for 60 min at 37°C, after which 60 μl of 10% (wt/vol) sodium dodecyl sulfate (15-min incubation at 65°C) and 0.1 mg of pronase (30 min incubation at 37°C) were added. After lysis, total DNA was precipitated by using 160 μl of 8 M LiCl and washed once in 70% (vol/vol) ethanol, and the final pellet resuspended in 50 μl of TE buffer containing 100 μg of RNase A/ml. PCR-based detection of *lanB* and *lanC* homologues was accomplished by using degenerate lantibiotic primer pairs derived from conserved amino acid tracts in six (streptin, pep5, nisin, epidermin, epicidin, and subtilin) and seven (the above plus salivaricin A) other lantibiotics, respectively: LanBFwd-LanBRev and LanCFwd-LanCRev (Table 2), which were kindly provided by P. A. Wescombe. Briefly, *S. uberis* 42 genomic DNA with an initial mix containing 10× buffer, and primers were incubated at 100°C for 3 min and cooled to 40°C. A second mix containing nucleotides and *Taq* DNA Polymerase (Roche Diagnostics GmbH, Mannheim, Germany) was added, and PCR conducted in a Mastercycler apparatus (Eppendorf), using an initial incubation step of 72°C for 1 min, followed by annealing temperature of 40°C for 30 s, and an elongation step at 72°C for 1 min for 30 repetitive cycles. PCR products were excised from agarose gels and purified by using the QIAquick Gel Extraction Kit (QIAGEN) and were ligated into the vector pGEM-T (Promega Co., Madison, WI) prior to electroporation into *E. coli* DH10B cells according to the supplier's instructions. Potential transformants were screened for inserts on LB-Amp media by using blue-white colony selection (33). Plasmids were extracted from white colonies by using the QIAprep Spin Miniprep Kit (QIAGEN), and the inserts were sequenced, either at the Centre for Gene Research (University of Otago) or the Allan Wilson Centre Genome Service (Massey University, Palmerston North, New Zealand). Nucleotide sequence data was assembled and analyzed by using the Lasergene sequence analysis software package (DNASTAR, Inc., Madison, Wis.). Homology searches were carried out by using appropriate BLAST algorithms (1) available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Based on the sequences obtained for the *lanB* and *lanC* PCR products, inverse PCR primers were designed (Table 2). A 15.6-kb contiguous sequence encompassing the entire nisin U locus was assembled from products generated by inverse PCR (33). All inverse PCR experiments were conducted by using the Expand Long Template PCR Kit (Roche). Chromosomal DNA (obtained using a DNeasy Tissue Kit, QIAGEN) digested with either EcoRI, EcoRV, HindIII, NdeI, Sau3aI, SpeI, or XbaI (all supplied by Roche) and ligated with T4 ligase (Roche) was used as a template. The entire nisin U locus was obtained by successive rounds of primer walking and sequencing of PCR amplicons.

Distribution of nisin U in other *S. uberis* strains. Nucleotide primers (Invitrogen, Carlsbad, CA) used in the present study included one pair internal to the nisin U structural gene *nsuA* (NsuAFwd and NsuARev) and another pair internal to the putative immunity gene *nsuI* (NsuIFwd and NsuIRev) (Table 2). DNA extracted from 45 strains of *S. uberis* was applied to a nylon membrane (Hybond-N^+; Amersham Biosciences United Kingdom, Ltd., Bucks, United Kingdom) using a Dot Blot apparatus (Bio-Rad Laboratories, Hercules, Calif.) and probed with digoxigenin-dUTP (Roche)-labeled *nsuA* according to the method of Wescombe and Tagg (43).

The presence of *nsuA* and *nsuI* in other strains was also established by colony PCR, using a mix containing the appropriate primer pair, nucleotides, buffer, and HotMaster *Taq* (Eppendorf). A single colony of each strain to be tested was

TABLE 2. Oligonucleotides used in this study

Primer name	Sequence (5′–3′)	Location[a]
UbnPinvL	GGTAATATTGGGCTTTACAGTCATA	914–890
UbnPsauinvR	CAAGTAAATGGAACTGTACAAGATA	1542–1566
UbnPRinvL	GTTGCATAAATATCAATACTGTCTT	1672–1648
UbnPRinvR	TGCAATAATAGTGTAAAATAAAAG	1959–1982
UbnRSpeinvR	ACGGTGTTTGATTATGTACTGTTTGA	2272–2296
UbnRinvL	TTCCTATGGCGCTCTTCTCGTTTC	2377–2354
UbnRFwd	AATCGGATGCATTATTTCGTTCAA	2569–2592
FXbainvL2	TCAGTCCCCTCAGGCGTTTTG	4338–4318
UbnFXbainvL	CAGAACTGGATTTTTGTTTAAGCCATTCAG	4866–4837
NBXinvR2	GAAATGGAGGATAACAAATGAAC	5516–5538
NBXinvL2	CAGTCTTTAAGACAGAATGGAACC	6615–6592
NBinvL	CAAATACTACTAGTAATGAGCAAAAAC	7240–7214
NBXinvR	GTTAAAATAAAGGTAGTGGATAGATAGC	7276–7303
UbnABinvR	CTATAAATGGATACTAAAACTGAAG	7856–7882
UbnBTinvL	CTACAAGGTGTGAATCAAAAATAC	9433–9410
BNFwd	GAAATCGAAAGATATGGGGGATTAGAAAG	9832–9860
LanBinvR	GCCGAATTAAACTTAATGAATTGATGAC	9931–9904
LanBinvL	AATTATCAGACAGTTGTATTCATTTATCAG	10175–10204
BNRev	GTAAATTTACCCATGATTGTGCCTATTCC	10362–10334
TjoinFwd	GTAATTTTTATGCGTGTTGGTCAGC	10892–10916
TjoinRev	CGCTTCCTGGATAAATAAAAGAGACA	11435–11410
42seq2R	CTTCAAATTAAATTTCTTAACCGC	11740–11763
42seq2L	ATAAGTAATGATATTCTGGATG	12462–12441
UbnCHind3invR	CTAACAGGCTTTTATGTAGTATC	12672–12694
42LanCinvL	GACAATATTACACCAACACCAGCAAGACCG	12764–12793
CHind3invR3	GTCATATTCAAAACTAAAAGGCTATTCT	12791–12818
CinvL	AACCATTACTTCTTCTTCTGATGGATGCT	13067–13092
UbnIseqR	GGTTTCTTTGAGTTCGGATGTA	13746–13767
IseqR2	AGGAACCCTCCTCTTAAGACTTAGA	14344–14368
UbnIdownRev	TTATGGTTATCGGATTAGCCTTATTGT	15253–15227
LanBFwd	TATGATCGAGAARYAKAWAGATATGG[b]	
LanBRev	TTATTAIRCAIATGIAYDAWACT[b]	
LanCFwd	TAATTTAGGATWISYIMAYGG[b]	
LanCRev	ACCWGKIIIICCRTRRCACCA[b]	
NsuAFwd	GGATCTCATCAAAATCTCAAAGG	
NsuARev	CACAGGTTGCAGTTTTTAGTGGAC	
NsuIFwd	ATTCTGCTTAGTTGTTCTTCTTGGA	
NsuIRev	TCTCAACCGCTATATTACCTTTTTC	

[a] Locations of primers are based on GenBank accession number DQ146939. Primers numbered in reverse are located on the complementary strand.
[b] I, K, M, R, W, and Y are based on the IUPAC code, where I stands for any base, K = T/G, M = A/C, R = A/G, W = A/T, and Y = C/T.

added to the mix, and PCR analysis was conducted with an initial heating step of 94°C for 5 min. This was followed by 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 65°C for 30 s for 30 cycles.

Induction assays. *S. uberis* strain ATCC 27958 and *L. lactis* strain ATCC 1404 were grown overnight at 42°C in THB. The cells were harvested by centrifugation and washed four times in saline before resuspension in a volume of saline equivalent to the original culture volume. A total of 20 ml of tryptic soy broth (BD) was inoculated with 0.5% (vol/vol) of the washed cell suspension, and 180-μl aliquots were placed into the wells of a microtiter plate. A total of 18 wells were used for each strain (ATCC 27958 and ATCC 1404), grouped into three sets of six wells with each set corresponding to one lantibiotic preparation to be tested: (i) nisin A (Sigma-Aldrich Co., St. Louis, MO); (ii) nisin U; and (iii) the unrelated lantibiotic streptococcin A-FF22, a THB culture supernatant of *S. pyogenes* strain FF22 (14). Each lantibiotic preparation was adjusted to a titer of 1 AU/ml. Within each set, three wells were designated controls (or uninduced) and three as tests (i.e., the experiment was performed in triplicate). To each of the three test wells 20 μl of the appropriate lantibiotic preparation was added, and the microtiter plate incubated for 6 h at 37°C. After incubation, 20 μl of the corresponding lantibiotic preparation was added to each of the control wells. At this time samples were taken from each well (test and control) and assayed for inhibitory activity using a well diffusion method. Briefly, 50-μl samples were added to 6-mm diameter wells in BACa medium and allowed to air dry. The surface of the agar was sterilized by exposure to chloroform vapor for 30 min, followed by opening to expose it to the air for 30 min. A lawn culture of indicator *Micrococcus luteus* T-18 was then applied by swabbing from a 6-h THB culture. The presence of a zone of inhibition surrounding the samples from the test wells (absent in control wells) was taken to be an indication of induction of inhibitor production.

Cross-immunity assays. Sensitivity to nisin U was assessed in two ways. First, strains were cross-tested by the deferred antagonism method against the nisin U producer strain *S. uberis* ATCC 27958. Second, pure preparations of nisin U and of 1 μg of nisin A (Sigma-Aldrich)/ml were titrated against *S. uberis* 42, *S. uberis* ATCC 27958, *L. lactis* ATCC 1404, *L. lactis* A5, *L. lactis* T-21, and *M. luteus* T-18.

Nucleotide sequence accession number. The complete nucleotide sequence of the nisin U locus has been deposited in the GenBank database under accession number DQ146939.

RESULTS

Detection of broad-spectrum BLIS activity in *S. uberis*. P typing of a set of 253 independent isolates of *S. uberis* showed that 17 (7%) of these strains were particularly broadly active in that they inhibited all nine standard BLIS-indicator strains (i.e., P-type 777). In addition, 25 (10%) of the *S. uberis* inhibited all but indicator I-4 of this set, this equating to a P-type of 737. One of the P-type 777 isolates, strain 42, was selected for further characterization of its BLIS activity. The present study reports the characterization

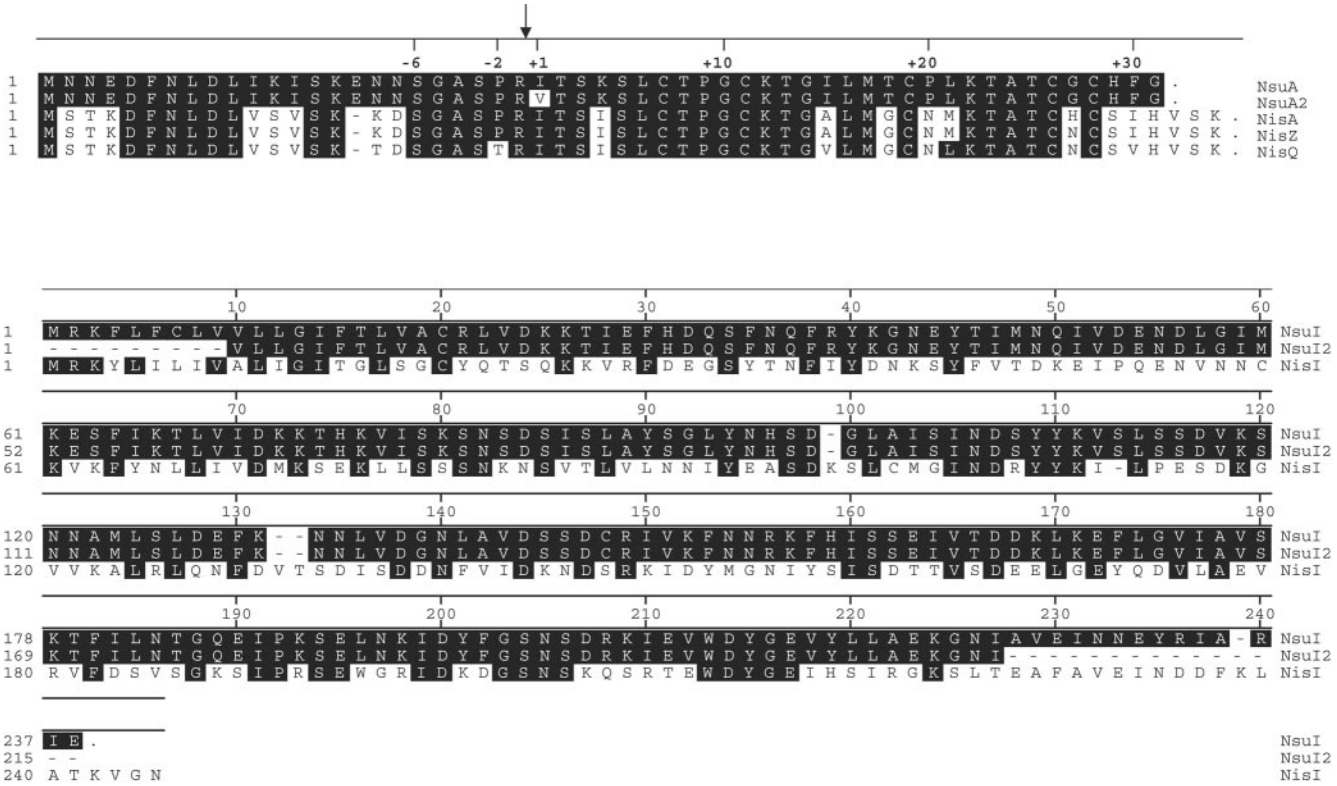

FIG. 1. Amino acid sequence of the prepeptide nisin U (NsuA) and variant in comparison to previously described nisin variants, and the putative immunity peptide, NsuI and its variant, to NisI. The proteolytic cleavage site is indicated by the arrow.

of a heat-stable peptide that accounts, in part, for the potent inhibitory activity of this bacterium.

Extraction, purification, and characterization of a heat-stable inhibitory peptide from *S. uberis* strain 42. The inhibitory activity of strain 42 was found to be largely cell-associated (85% of total activity), with only ca. 15% of the total inhibitory activity recovered from the supernatant fluid of Columbia broth cultures. It was decided to proceed with the acidified saline extract of the cell pellet for purification purposes, since this comprised the majority of the inhibitory activity and was also of a higher specific activity (containing relatively little of the culture medium). RP-HPLC of the cell extract yielded a single peak of inhibitory activity eluting at ca. 35% acetonitrile. The purified peptide (mass = 3,029.0 ± 0.3 Da as determined by ESI) yielded only an N-terminal isoleucine when sequenced by Edman degradation. Since it is known that the sequencing of lantibiotic-class bacteriocins is often blocked by the presence of dehydro-residues (23), the peptide of strain 42 was first derivatized by treatment with 1-propanethiol and then sequenced to yield: I-X-X-K-X-L-X-X-P-G-X-K-X-G-I-L-M-X-X-P. The purified peptide (at pH 2) was stable to heating at 100°C for 60 min and showed no loss of activity upon storage at 4°C for 1 year.

Identification of the structural gene. Based on the above characteristics, we hypothesized that the inhibitory peptide was a lantibiotic, and this was supported by the detection by PCR of both *lanB* and *lanC* homologues in strain 42. Sequencing of these products and subsequent primer walking disclosed a 15.6-kb lantibiotic-like locus. Upstream of the *lanB* homologue is an open reading frame (ORF) of 168 bp with strong homology to the structural genes known to encode the nisin A, Z, and Q prepeptides. The putative structural gene, *nsuA*, encodes a prepeptide, the sequence of which is shown in Fig. 1. The propeptide-encoding component of *nsuA* is consistent with the N-terminal sequence of the purified peptide from strain 42 (Fig. 1). While the "X" residues correspond to either serine, threonine, or cysteine in the prepeptide, often in mature biologically active lantibiotics, these particular amino acids have been converted to either lanthionine, methyllanthionine, or didehydro residues. The predicted mass for the nisin U propeptide deduced from the *nsuA* sequence is 3,172.6 Da, but after subtracting the mass of eight water molecules to account for dehydration of the six threonine and two serine residues in this part of the molecule, a value of 3,028.6 is obtained, which correlates closely with the mass (3,029 Da) obtained by MS. This indicates that every serine and threonine residue is probably either in the dehydrated state or incorporated into lanthionine or methyllanthionine residues. Due to its close structural and sequence similarity to nisin A, the inhibitory agent produced by *S. uberis* strain 42 was consequently designated nisin U.

Sequence analysis of the nisin U locus. Sequencing of the entire 15.6-kb nisin U locus showed it to comprise 11 ORFs (Fig. 2). Sequence analysis and BLAST homologies indicated these are likely to be involved in lantibiotic biosynthesis, regulation and immunity and were designated *nsuP*, *nsuR*, *nsuK*, *nsuF*, *nsuE*, *nsuG*, *nsuA*, *nsuB*, *nsuT*, *nsuC*, and *nsuI*. The sizes

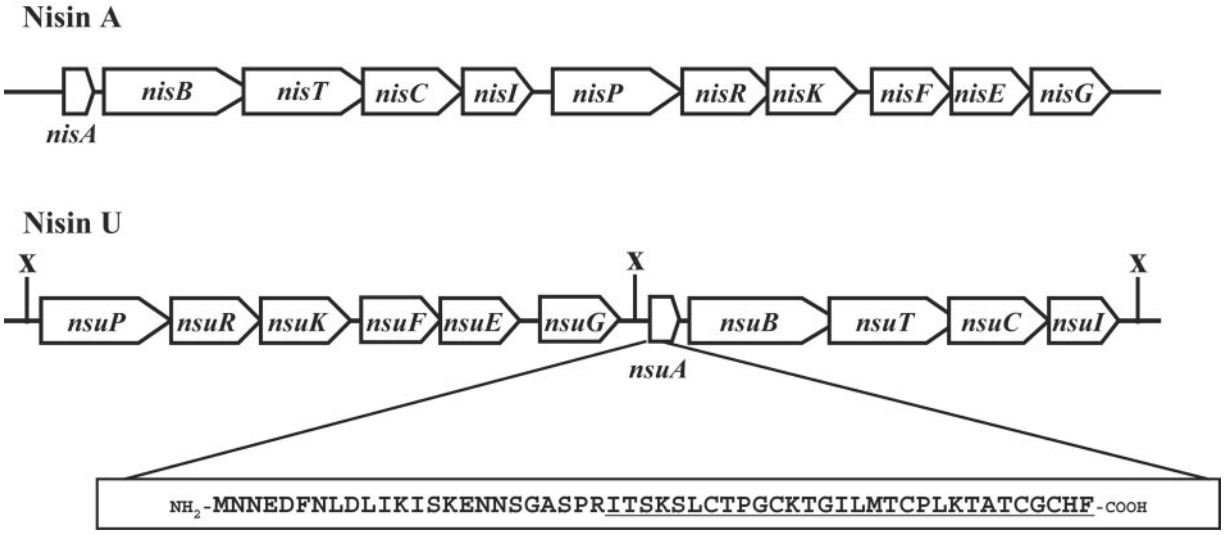

FIG. 2. Organization of the nisin U locus (bottom) in comparison to the nisin A locus (top). The deduced nisin U prepeptide encoded by *nsuA* is also shown, with the propeptide component indicated by underlining. The locations of regions with homologies to transposases are indicated by the "X" symbols.

of the gene products and their putative functions based on BLAST homologies are presented in Table 3.

Evaluation of the distribution and composition of the nisin U locus. From the 45 strains tested by dot blot and colony PCR, 10 were positive for *nsuA*: six *S. uberis* strains of P-type 737 (ATCC 27958, D535, D536, 71-612, RF1384, and NY41) and four *S. uberis* strains of P-type 777 (42, J4, J115, and B10a) were found to have an *nsuA* homologue. Of the *nsuA*-negative strains, 7 were P-type 000 and the remainder displayed 13 different P-type patterns. Although the *nsuA* nucleotide sequences in general appeared to be highly conserved in *S. uberis*, a putative propeptide variant (Ile1Val) named NsuA2 was detected in strains D536 and 71-612 (Fig. 1). Another strain (RF1384) had one predicted amino acid difference in its leader peptide. Putative immunity genes (*nsuI*) were detected in all strains harboring *nsuA* and its homologues. A variant of the putative product of *nsuI* was found (NsuI2) in strains D535 and D536 (Fig. 1). Analysis of the nisin U locus in *S. uberis* 42 revealed a 742-bp region between the end of *nsuG* and the start of *nsuA*, displaying no homologies to other known genes commonly found in lantibiotic loci. Approximately 400 bp in the middle of this region yielded BLAST homologies to transposases. Weak homologies to insertion sequences were also found to the sequences on either side of the nisin U locus.

Inhibitory spectrum of nisin U. When assayed against the set of nine standard indicator bacteria, purified nisin U exhibited a spectrum of inhibitory activity closely similar to that of similarly prepared extracts from *S. uberis* strain ATCC 27958 (18) and *L. lactis* strain ATCC 1404 (producer of nisin A) (Table 4). However, there were some notable differences in the activities of nisin A and nisin U against some indicator strains, with nisin A acting to a relatively greater extent than nisin U on *S. pyogenes* (strains I-2, I-5, I-7, and I-8) and *L. lactis* (strain) (I-6) (Table 4). The spectrum of activity of both of these preparations against the standard indicators (Table 4) corresponds to a P-type profile of 737 (i.e., no inhibition of I-4). Since *S. uberis* strain ATCC 27958 demonstrates this P-type (39), it appears that the inhibitory activity of this strain may be

TABLE 3. Characteristics of predicted proteins specified by the *S. uberis* 42 *nsu* gene cluster

Gene	Gene size (bp)	Deduced protein characteristics			
		Size (kDa)	pI	Homology[a] (% identity, % similarity)	Putative function
nsuP	1,386	51.4	4.8	Serine protease, NisP (37, 58)	Proteolytic cleavage of leader peptide
nsuR	699	27	5.4	Response regulator, NisR (64, 86)	Lantibiotic regulation
nsuK	1,347	51.6	5.4	Histidine kinases, NisK (42, 63)	Lantibiotic regulation
nsuF	693	25.3	5.7	ABC transporters, NisF (65, 82)	Immunity
nsuE	762	28.9	9.0	Membrane protein, NisE (39, 59)	Immunity
nsuG	693	26.5	9.3	Membrane protein, NisG (35, 53)	Immunity
nsuA	168	5.9	8.1	Lantibiotic prepeptide	Lantibiotic nisin U
nsuB	2,976	117.1	6.1	Lantibiotic biosynthesis protein, NisB (42, 63)	Lantibiotic biosynthesis, dehydratase
nsuT	1,797	68.9	8.9	ABC transporter, NisT (54, 72)	Lantibiotic translocator/transporter
nsuC	1,278	48.8	7.9	Lantibiotic biosynthesis protein, NisC (34, 54)	Thioether formation
nsuI	717	27.3	6.0	Nisin immunity protein, NisI (32, 55)	Immunity

[a] Values obtained through BLAST searches.

TABLE 4. Comparison of inhibitory activities and specificities of preparations of nisin A and nisin U

BLIS indicator strain	Titer against BLIS indicator of nisin extract from strain		
	S. uberis 42[a]	*S. uberis* ATCC 27958[a]	*L. lactis* ATCC 1404[b]
I-1	512	512	512
I-2	4	4	32
I-3	1	1	2
I-4	0	0	0
I-5	2	2	8
I-6	8	8	64
I-7	4	4	32
I-8	2	2	8
I-9	1	1	1

[a] Producer of nisin U.
[b] Producer of nisin A.

solely attributable to nisin U. When tested for its inhibitory spectrum in deferred antagonism tests, strain ATCC 27958 was active against a variety of other streptococci, including most *S. pyogenes*, *S. salivarius*, *S. uberis*, *S. agalactiae*, and *S. dysgalactiae*. On the other hand, there was, with the exception of *Staphylococcus cohnii* and *Staphylococcus simulans*, relatively little activity against staphylococci, *S. mutans*, lactobacilli, or gram-negative bacteria (Table 5). Interestingly, a nisin U preparation had a relatively higher titer on *S. uberis* strain E, but a lower titer on *L. lactis* T-21, than did a nisin A preparation adjusted to have similar activity to that of nisin U preparation against *M. luteus* (Table 6).

Induction of lantibiotic production. The production of nisin U by *S. uberis* ATCC 27958 and of nisin A by *L. lactis* ATCC 1404 was induced by addition of either nisin A or nisin U to the washed (lantibiotic depleted) producer cells. No induction of nisin production was obtained on addition of the unrelated lantibiotic streptococcin A-FF22.

TABLE 5. Spectrum of inhibitory activity of *S. uberis* ATCC 27958[a] in deferred antagonism tests

Species tested	No. strains inhibited/no. strains tested
Streptococcus pyogenes	10/11
Streptococcus salivarius	0/7
Streptococcus uberis	20/26
Streptococcus agalactiae	2/4
Streptococcus dysgalactiae	3/4
Enterococcus faecalis	0/3
Staphylococcus aureus	0/2
Staphylococcus simulans	1/1
Staphylococcus cohnii	1/1
Lactococcus lactis	1/3
Lactobacillus acidophilus	1/1
Lactobacillus casei	0/1
Streptococcus rattus	0/2
Streptococcus mitis	2/2
Streptococcus mutans	0/7
Escherichia coli	0/4
Enterobacter aerogenes	0/1
Salmonella enterica serovar Typhimurium	0/1

[a] Producer of nisin U only.

TABLE 6. Demonstration of cross-immunity of nisin producers to both nisin A and nisin U

Indicator strain	Inhibitor titer (AU/ml) of extract from strain:	
	ATCC 27958[a]	ATCC 1404[b]
M. luteus T-18	512	512
S. uberis ATCC 27958[a]	0	0
S. uberis 42[a]	0	0
S. uberis E	8	2
L. lactis ATCC 1404[b]	0	0
L. lactis A5[c]	0	0
L. lactis T-21	8	64

[a] Producer of nisin U.
[b] Producer of nisin A.
[c] Producer of nisin Z.

Cross-immunity. All strains harboring *nsuA* and *nsuI* demonstrated resistance to *S. uberis* ATCC 27958 when evaluated as indicator strains in deferred antagonism tests and also when tested for sensitivity to nisin U preparations. In addition, the nisin U producer strain ATCC 27958, the nisin A producer strain ATCC 1404, and the nisin Z producer strain A5 were also resistant to preparations of either nisin U or nisin A (Table 6). These findings support the molecular data and indicate that the immunity peptides encoded by the nisin U, nisin A, and nisin Z loci confer protection against both nisin A and nisin U.

DISCUSSION

The production of lantibiotics is widespread among gram-positive bacteria (32). As more lantibiotics are detected, it is becoming increasingly clear that naturally occurring variants occur quite commonly, with some of these differing in only a single amino acid residue (for example, nisin A and nisin Z [Asn27-nisin]), whereas others involve several amino acid differences such as between epidermin and mutacin B-Ny266 (Phe1, Lys2, Trp4, Dha5, Phe6-epidermin) (24). It has been recommended that "by definition" natural lantibiotic variants should have only "a few" amino acid differences, essentially the same ring pattern, and that cross-immunity should be exhibited between their respective producing strains (32).

Nisin U, so called because of its original isolation from a strain of *S. uberis*, is the first variant of nisin to be characterized from a species other than *Lactococcus lactis*. It is a 31-amino-acid lantibiotic exhibiting 78% identity (82% similarity) to nisin A and Z and 82% identity (85% similarity) to nisin Q.

Although the 11 ORFs of the nisin U locus in *S. uberis* strain 42 are closely similar to their nisin A counterparts, their arrangement in the host bacteria is somewhat different (Fig. 2). The striking difference is that *nsuP*, *nsuRK*, and *nsuFEG* are located upstream of *nsuA* in the nisin U locus, whereas in the nisin A locus *nisPRKFEG* are downstream of *nisI* (Fig. 2). The presence of a region with homologies to transposases in the 742-bp noncoding region between *nsuG* and *nsuA* and in the sequences either side of the *nsu* locus indicates that *S. uberis* 42 may have acquired the locus from another organism and that some rearrangement of the genes may have occurred during this process. Although there have been several reports of transposon associations with the nisin A and nisin Z operons (9, 20, 28, 41), none of the transposon

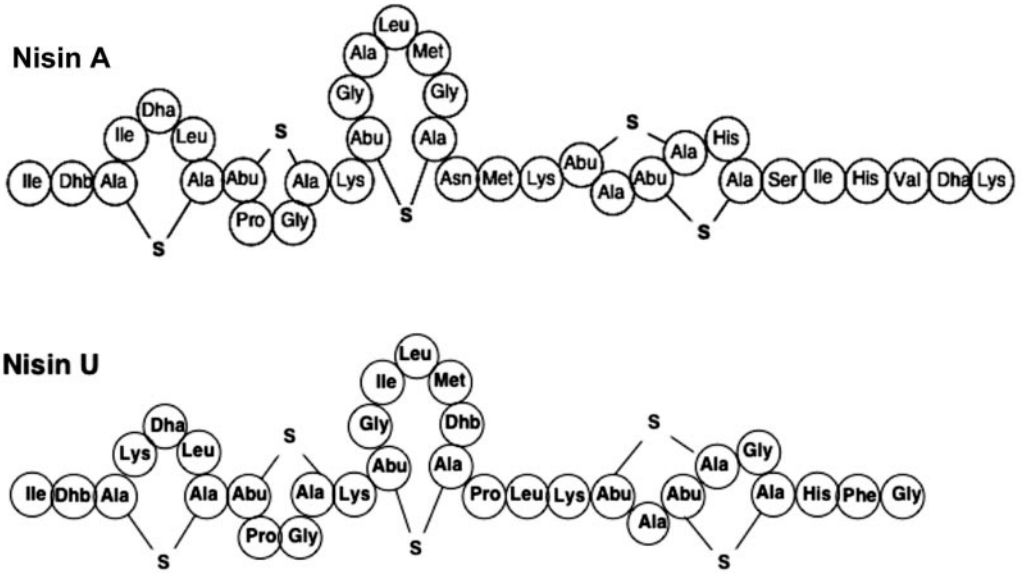

FIG. 3. Putative ring structure of nisin U (bottom) based on sequence similarities to nisin A (top). Abu, aminobutyric acid; Dha, didehydroalanine; Dhb, didehydrobutyrine.

remnants detected in strain 42 bear any similarity to those previously described in *L. lactis* host strains.

Nisin U is a member of the type A lantibiotics (elongated and amphiphilic molecules with a molecular mass in the range of 2 to 5 kDa) (32) and also has the properties of the type AI lantibiotics, with a leader peptide containing the characteristic FNLD box, a serine at −6, and a proline at −2 (22). In view of the close identity of the nisin U and nisin A amino acid sequences, the apparent similarities in their activity spectra, and their cross-immunity and cross-induction capabilities, it seems that these two peptides probably have identical heterocyclic ring structures (Fig. 3). The nisin A and nisin Z molecules consist of N-terminal and C-terminal domains connected by methionine at position 21, which acts as a flexible hinge region (40). In nisin U, the methionine is substituted by leucine, as is also found in nisin Q (45) and the nisin-like lantibiotic subtilin (2). A potentially significant difference in nisin U is the presence of a proline in position 20, in place of the asparagine in nisins A, Z, and Q. An example of the possible effect of proline in this region is in epicidin 280, which has 75% sequence similarity to Pep5 (12). Epicidin 280 has a decreased spectrum of activity compared to Pep5, and this is thought to be due to the proline at position 16 (part of the flexible hinge region in Pep5), which may contribute to decreased flexibility in the central part of the epicidin peptide (12). In other studies, a nisin mutant with two proline substitutions in the flexible hinge region (N20P/M21P-nisin) was found to be inactive (44). The single proline substitution in nisin U may therefore contribute to its different target organism specificity compared to nisin A. Nisin U appears to have a Dhb residue in position 18 (as does subtilin), but this change is not considered likely to significantly affect the overall conformational flexibility of the molecule (8). Interestingly, nisin U is three amino acids shorter than the other forms of nisin. However, nisin mutation studies have shown that nisin1-32 (lacking the last two amino acids) is just as active as the full-length nisin (7, 29).

Immunity to nisin seems to depend upon expression of both *nisI* and *nisFEG* (27). However, it has been demonstrated that *nisI* mutants show more pronounced sensitivity to exogenous nisin than *nisF*, *nisE*, or *nisG* mutants (35), indicating a more significant role for NisI in immunity. Within the nisin U locus, *nsuI* encodes a peptide with 55% sequence similarity to NisI and, like NisI (20) and SpaI (17), it also has a lipoprotein signal sequence. This finding is interesting because even though nisin and subtilin share 60% sequence similarity, they do not exhibit cross-immunity, indicating the highly specific nature of the interactions between these lantibiotics and their respective immunity proteins (22) and highlighting the importance of immunity peptide compatibility in order for cross-resistance to occur. To date, there has only been one reported case of cross-immunity between apparently unrelated lantibiotics: that between epicidin 280 and Pep5, which are believed to interact with functionally related, through sequentially diverse immunity peptides (12).

Nisin A biosynthesis is known to be primarily regulated by the *nisRK*-encoded two-component signal transduction system, in which *nisA* transcription is autoregulated by fully modified nisin accumulating at critical concentrations at the external surface of the producer cells (19). The streptococcal lantibiotic streptin also appears to act as the specific inducer of its own production (43). In the present study, we found that either nisin A or nisin U could function to induce either nisin A or nisin U production. In contrast, the unrelated lantibiotic streptococcin A-FF22 failed to induce production of either type of nisin. A putative nis-box was detected upstream of *nsuP*, and this is similar to that shown to have a role in nisin A autoregulation (16).

The nisin U structural gene *nsuA*, and the genes on either side of it (*nsuB* and *nsuG*) were detected in a total of 10

independent isolates of *S. uberis* (including strain 42) (data not shown). Natural variants of nisin U were found in two of these *S. uberis*. The only amino acid difference in the variant propeptide was due to a valine substitution for the isoleucine in position +1. Since these are both nonpolar, hydrophobic amino acids, this change is considered unlikely to bring about any substantial structural change in the peptide.

Assay of a purified nisin U preparation against the set of nine standard BLIS-detector strains indicated inhibitory activity consistent with a P-type pattern 737 in deferred antagonism tests. *S. uberis* strain ATCC 27958, which also exhibits this P type was shown to harbor the nisin U locus, thus supporting the correlation between P-type 737 BLIS activity and nisin U production in *S. uberis*.

In the present study, we report (i) the first characterization of a bacteriocin locus in *S. uberis* and (ii) that lantibiotics of the nisin family can be produced by bacteria other than of the genus *Lactococcus*. Nisin U exhibits the typical characteristics of lantibiotics, including remarkable heat stability, a large content of modified amino acids, and an operon comprising genes necessary for biosynthesis, regulation, and host-cell specific immunity. Within the nisin U locus there is a 400-bp region encoding putative transposase-like elements, indicating a possible mechanism for the acquisition of this locus by *S. uberis*.

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REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
2. **Banerjee, S., and J. N. Hansen.** 1988. Structure and expression of a gene encoding the precursor of subtilin, a small protein antibiotic. J. Biol. Chem. **263:**9508–9514.
3. **Bierbaum, G., F. Gotz, A. Peschel, T. Kupke, M. van de Kamp, and H.-G. Sahl.** 1996. The biosynthesis of the lantibiotics epidermin, gallidermin, Pep5 and epilancin K7. Antonie Leeuwenhoek **69:**119–127.
4. **Blowey, R., and P. Edmondson.** 1995. Mastitis control in dairy herds: an illustrated and practical guide. Farming Press Books, Ipswich, United Kingdom.
5. **Broadbent, J. R., C. Chou, K. Gillies, and J. K. Kondo.** 1989. Nisin inhibits several gram-positive, mastitis-causing pathogens. J. Dairy Sci. **72:**3342–3345.
6. **Buddle, B. M., J. R. Tagg, and M. J. Ralston.** 1988. Use of an inhibitor typing scheme to study the epidemiology of *Streptococcus uberis* mastitis. N. Z. Vet. J. **36:**115–119.
7. **Chan, W. C., B. W. Bycroft, L.-Y. Lian, and G. C. K. Roberts.** 1989. Isolation and characterisation of two degradation products derived from the peptide antibiotic nisin. FEBS Lett. **252:**29–36.
8. **Chatterjee, C., M. Paul, L. Xie, and W. A. van der Donk.** 2005. Biosynthesis and mode of action of lantibiotics. Chem. Rev. **105:**633–684.
9. **de Vos, W. M., O. P. Kuipers, J. R. van der Meer, and R. J. Siezen.** 1995. Maturation pathway of nisin and other lantibiotics: posttranslationally modified antimicrobial peptides exported by gram-positive bacteria. Mol. Microbiol. **17:**427–437.
10. **Guder, A., I. Wiedemann, and H.-G. Sahl.** 2000. Post-translationally modified bacteriocins: the lantibiotics. Biopolymers **55:**62–73.
11. **Hardie, J. M.** 1986. Genus *Streptococcus* Rosenbach 1884, 22AL, p. 1043–1071. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore, Md.
12. **Heidrich, C., U. Pag, M. Josten, J. Metzger, R. W. Jack, G. Bierbaum, G. Jung, and H.-G. Sahl.** 1998. Isolation, characterization, and heterologous expression of the novel lantibiotic epicidin 280 and analysis of its biosynthetic gene cluster. Appl. Environ. Microbiol. **64:**3140–3146.
13. **Hubbard, M. J., N. J. McHugh, and D. L. Carne.** 2000. Isolation of ERp29, a novel endoplasmic reticulum protein, from rat enamel cells. Eur. J. Biochem. **267:**1945–1956.
14. **Jack, R. W., and J. R. Tagg.** 1991. Isolation and partial structure of streptococcin A-FF22, p. 171–179 *In* G. Jung and H.-G. Sahl (ed.), Nisin and novel lantibiotics. Escom Publishers, Leiden, The Netherlands.
15. **Jayarao, B. M., S. P. Oliver, J. R. Tagg, and K. R. Matthews.** 1991. Genotypic and phenotypic analysis of *Streptococcus uberis* isolated from bovine mammary secretions. Epidemiol. Infect. **107:**543–555.
16. **Kleerebezem, M.** 2004. Quorum sensing control of lantibiotic production: nisin and subtilin autoregulate their own biosynthesis. Peptides **25:**1405–1414.
17. **Klein, C., and K. D. Entian.** 1994. Genes involved in self-protection against the lantibiotic subtilin produced by *Bacillus subtilis* ATCC 6633. Appl. Environ. Microbiol. **60:**2793–2801.
18. **Klesse, N. A.** 2001. Purification and partial characterisation of a novel bacteriocin produced by *Streptococcus uberis* strain ATCC 27958. MSc. thesis. University of Otago, Dunedin, New Zealand.
19. **Kuipers, O. P., M. M. Beerthuyzen, P. G. de Ruyter, E. J. Luesink, and W. M. de Vos.** 1995. Autoregulation of nisin biosynthesis in *Lactococcus lactis* by signal transduction. J. Biol. Chem. **270:**27299–27304.
20. **Kuipers, O. P., M. M. Beerthuyzen, R. J. Siezen, and W. M. De Vos.** 1993. Characterization of the nisin gene cluster *nisABTCIPR* of *Lactococcus lactis*. Requirement of expression of the *nisA* and *nisI* genes for development of immunity. Eur. J. Biochem. **216:**281–291.
21. **Leigh, J. A.** 1999. *Streptococcus uberis*: a permanent barrier to the control of bovine mastitis? Vet. J. **157:**225–238.
22. **McAuliffe, O., R. P. Ross, and C. Hill.** 2001. Lantibiotics: structure, biosynthesis and mode of action. FEMS Microbiol. Rev. **25:**285–308.
23. **Meyer, H. E., M. Heber, B. Eisermann, H. Korte, J. W. Metzger, and G. Jung.** 1994. Sequence analysis of lantibiotics: chemical derivatization procedures allow a fast access to complete Edman degradation. Anal. Biochem. **223:**185–190.
24. **Mota-Meira, M., C. Lacroix, G. LaPointe, and M. C. Lavoie.** 1997. Purification and structure of mutacin B-Ny266: a new lantibiotic produced by *Streptococcus mutans*. FEBS Lett. **410:**275–279.
25. **Mulders, J. W., I. J. Boerrigter, H. S. Rollema, R. J. Siezen, and W. M. de Vos.** 1991. Identification and characterization of the lantibiotic nisin Z, a natural nisin variant. Eur. J. Biochem. **201:**581–584.
26. **Nes, I. F., and J. R. Tagg.** 1996. Novel lantibiotics and their pre-peptides. Antonie Leeuwenhoek **69:**89–97.
27. **Ra, R., M. M. Beerthuyzen, W. M. de Vos, P. E. Saris, and O. P. Kuipers.** 1999. Effects of gene disruptions in the nisin gene cluster of *Lactococcus lactis* on nisin production and producer immunity. Microbiology **145:**1227–1233.
28. **Rauch, P. J., M. M. Beerthuyzen, and W. M. de Vos.** 1994. Distribution and evolution of nisin-sucrose elements in *Lactococcus lactis*. Appl. Environ. Microbiol. **60:**1798–1804.
29. **Rollema, H. S., J. W. Metzger, P. Both, O. P. Kuipers, and R. J. Siezen.** 1996. Structure and biological activity of chemically modified nisin A species. Eur. J. Biochem. **241:**716–722.
30. **Ryan, M. P., W. J. Meaney, R. P. Ross, and C. Hill.** 1998. Evaluation of lacticin 3147 and a teat seal containing this bacteriocin for inhibition of mastitis pathogens. Appl. Environ. Microbiol. **64:**2287–2290.
31. **Sahl, H.-G., and G. Bierbaum.** 1998. Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria. Annu. Rev. Microbiol. **52:**41–79.
32. **Sahl, H.-G., R. W. Jack, and G. Bierbaum.** 1995. Biosynthesis and biological activities of lantibiotics with unique posttranslational modifications. Eur. J. Biochem. **230:**827–853.
33. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
34. **Schnell, N., K. D. Entian, U. Schneider, F. Gotz, H. Zahner, R. Kellner, and G. Jung.** 1988. Prepeptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings. Nature **333:**276–278.
35. **Siegers, K., and K. D. Entian.** 1995. Genes involved in immunity to the lantibiotic nisin produced by *Lactococcus lactis* 6F3. Appl. Environ. Microbiol. **61:**1082–1089.
36. **Siezen, R. J., O. P. Kuipers, and W. M. de Vos.** 1996. Comparison of lantibiotic gene clusters and encoded proteins. Antonie Leeuwenhoek **69:**171–184.
37. **Steen, M. T., Y. J. Chung, and J. 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.
38. **Tagg, J. R., and L. V. Bannister.** 1979. "Fingerprinting" beta-hemolytic streptococci by their production of and sensitivity to bacteriocin-like inhibitors. J. Med. Microbiol. **12:**397–411.
39. **Tagg, J. R., and L. G. Vugler.** 1986. An inhibitor typing scheme for *Streptococcus uberis*. J. Dairy Res. **53:**451–456.
40. **Van de Ven, F. J., H. W. Van den Hooven, R. N. Konings, and C. W. Hilbers.** 1991. NMR studies of lantibiotics. The structure of nisin in aqueous solution. Eur. J. Biochem. **202:**1181–1188.
41. **van der Meer, J. R., J. Polman, M. M. Beerthuyzen, R. J. Siezen, O. P. Kuipers, and W. M. De Vos.** 1993. Characterization of the *Lactococcus lactis* nisin A operon genes *nisP*, encoding a subtilisin-like serine protease involved

in precursor processing, and *nisR*, encoding a regulatory protein involved in nisin biosynthesis. J. Bacteriol. **175:**2578–2588.

42. **Walk, T. B., R. Sussmuth, C. Kempter, V. Gnau, R. W. Jack, and G. Jung.** 1999. Identification of unusual amino acids in peptides using automated sequential Edman degradation coupled to direct detection by electrospray-ionization mass spectrometry. Biopolymers **49:**329–340.
43. **Wescombe, P. A., and J. R. Tagg.** 2003. Purification and characterization of streptin, a type A1 lantibiotic produced by *Streptococcus pyogenes*. Appl. Environ. Microbiol. **69:**2737–2747.
44. **Wiedemann, I., E. Breukink, C. van Kraaij, O. P. Kuipers, G. Bierbaum, B. de Kruijff, and H.-G. Sahl.** 2001. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J. Biol. Chem. **276:**1772–1779.
45. **Zendo, T., M. Fukao, K. Ueda, T. Higuchi, J. Nakayama, and K. Sonomoto.** 2003. Identification of the lantibiotic nisin Q, a new natural nisin variant produced by *Lactococcus lactis* 61-14 isolated from a river in Japan. Biosci. Biotechnol. Biochem. **67:**1616–1619.