

Properties of Nisin Z and Distribution of Its Gene, *nisZ*, in *Lactococcus lactis*

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Two natural variants of the lantibiotic nisin that are produced by *Lactococcus lactis* are known. They have a similar structure but differ in a single amino acid residue at position 27: histidine in nisin A and asparagine in nisin Z (J. W. M. Mulders, I. J. Boerriqter, H. S. Rollema, R. J. Siezen, and W. M. de Vos, *Eur. J. Biochem.* 201:581–584, 1991). The nisin variants were purified to apparent homogeneity, and their biological activities were compared. Identical MICs of nisin A and nisin Z were found with all tested indicator strains of six different species of gram-positive bacteria. However, at concentrations above the MICs, with nisin Z the inhibition zones obtained in agar diffusion assays were invariably larger than those obtained with nisin A. This was observed with all tested indicator strains. These results suggest that nisin Z has better diffusion properties than nisin A in agar. The distribution of the nisin variants in various lactococcal strains was determined by amplification of the nisin structural gene by polymerase chain reaction followed by direct sequencing of the amplification product. In this way, it was established that the *nisZ* gene for nisin Z production is widely distributed, having been found in 14 of the 26 *L. lactis* strains analyzed.

Various gram-positive bacteria produce small peptides with antimicrobial activity that share the presence of the unusual amino acids lanthionine and β -methylanthionine and hence are termed lantibiotics (13, 27). Several of these lantibiotics have been characterized at the protein and gene levels, including subtilin, made by *Bacillus subtilis* (2); epidermin (27) and Pep5 (15), which are synthesized by *Staphylococcus* spp.; and nisin, produced by *Lactococcus lactis* (4, 9). Comparison of the protein and gene sequences of the lantibiotics has shown that they are ribosomally synthesized as prepeptides containing an unusual and structurally homologous leader sequence that may be involved in posttranslational modification processes (4, 13, 27). Evidence of the existence of some of these modification reactions has recently been provided by the isolation of intracellular processing intermediates of Pep5 (30).

Nisin is a heat-stable peptide that was first described to be produced by a single strain of group N streptococci, now known as lactococci (17). It is widely used as a natural food preservative since it shows antimicrobial activity against various gram-positive bacteria, including strains of *Clostridium*, *Listeria*, and *Bacillus* spp. and most lactic acid bacteria (12). Initial biochemical studies showed that nisin isolated from a single producer strain consists of a mixture of closely related peptides with different antimicrobial activities (3). The structure of the 34-residue main component with the highest activity, nisin A, has been determined (9). Recent genetic studies have shown that a single structural gene for nisin production (designated *nisA* [14]) is present in four different nisin-producing *L. lactis* strains (4, 7, 14, 19) and is located on large conjugative transposons in several strains (11, 19). The deduced sequence of the 34-amino-acid structural region of the nisin precursor confirms the previously determined structure of nisin A (9), substantiates the postulated posttranslational modification reactions (13), and indi-

cates that the initially identified forms of nisin may be attributed to degradation of matured nisin (6) or to different degrees of posttranslational modification.

In the course of screening naturally occurring nisin overproducers, we identified *L. lactis* NIZO 22186 which had been isolated from a dairy product. This strain produced high levels of an antimicrobial activity similar but not identical to that of nisin. Subsequent biochemical and genetic characterization showed that strain NIZO 22186 produces a natural nisin variant encoded by the *nisZ* gene (18). The *nisA* and *nisZ* genes differ in a single nucleotide substitution in the structural region. As a consequence, the *nisZ*-encoded lantibiotic, designated nisin Z, contains an asparagine at position 27 instead of a histidine as in nisin A (18). In this report, we compare relevant physicochemical and biological properties of nisin Z with those of its variant, nisin A, and show that the *nisZ* gene is widely distributed in naturally occurring nisin-producing *L. lactis* strains.

(A preliminary account of some of these results was reported at the First International Workshop on Lantibiotics, 1991, Bad Honnef, Germany.)

MATERIALS AND METHODS

Strains, media, and bioassays of nisin analogs. *L. lactis* strains screened for the production of lantibiotics and their origins are listed in Table 1. *L. lactis* cells were routinely grown at 30°C in media based on M17 medium (Oxoid Ltd., Basingstoke, England) supplemented with 0.5% lactose, glucose, or sucrose when appropriate. For production of lantibiotics, *L. lactis* strains were grown as described previously (18). *Micrococcus flavus* DSM 1719 (obtained from the German Collection of Microorganisms, Braunschweig, Germany) was grown under aerobic conditions in nutrient broth (Difco Laboratories, Detroit, Mich.). The following strains were obtained from the Netherlands Institute for Dairy Research (NIZO) culture collection and handled as indicated: *Bacillus cereus* (strains P7 and C5) and *Listeria*

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TABLE 1. *L. lactis* strains used in this study, their origin, and the presence of *nisA* or *nisZ* genes as deduced from sequence analysis of PCR-amplified *nis* genes

Strain	Type of <i>nisin</i> gene	Source (reference) ^a
NIZO R5	<i>nisA</i>	NIZO collection (19)
INRA 1	<i>nisA</i>	G. Limsowtin, INRA
INRA 2	<i>nisA</i>	G. Limsowtin, INRA
INRA 3	<i>nisA</i>	G. Limsowtin, INRA
INRA 4	<i>nisA</i>	G. Limsowtin, INRA
INRA 5	<i>nisA</i>	G. Limsowtin, INRA
INRA 6	<i>nisA</i>	G. Limsowtin, INRA
NP4G	<i>nisA</i>	L. Topisirovic, Institute for Molecular Genetics, Belgrade, Yugoslavia
NZI	<i>nisA</i>	M. Nuti, University of Padua, Padua, Italy
NCDO 2111	<i>nisA</i>	NCDO
S1	<i>nisA</i>	J. Bardowski, INRA
ILC13	<i>nisA</i>	G. Giraffa, ILC
NIZO 22186	<i>nisZ</i>	NIZO collection (18)
NIZO N9	<i>nisZ</i>	NIZO collection
ATCC 7962	<i>nisZ</i>	Obtained as NCDO 2054 from NCDO
NCDO 2118	<i>nisZ</i>	NCDO
NCDO 2597	<i>nisZ</i>	Obtained as LMG 8514 from B. Pot, LMG Culture Collection, Ghent, Belgium
NCDO 2091	<i>nisZ</i>	Obtained as LMG 8526 from B. Pot, LMG
NCK400	<i>nisZ</i>	T. R. Klaenhammer, NCSU (10)
LJN80	<i>nisZ</i>	T. R. Klaenhammer, NCSU (10)
ILC11	<i>nisZ</i>	G. Giraffa, ILC
ILC19	<i>nisZ</i>	G. Giraffa, ILC
ILC126	<i>nisZ</i>	G. Giraffa, ILC
ILCSL5	<i>nisZ</i>	G. Giraffa, ILC
ILCSL20	<i>nisZ</i>	G. Giraffa, ILC
I01	<i>nisZ</i>	A. Ishizaki, Kyushu University, Higashi-ku, Japan

^a INRA, Institut National de Recherche Agronomique, Jouy en Josas, France; NCDO, National Collection of Dairy Organisms; ILC, Istituto Sperimentale Lattiero-Caseario, Lodi-Milan, Italy; NCSU, North Carolina State University.

monocytogenes (strains 13 and 669), grown in Trypticase soy broth (Difco Laboratories) at 30°C under aerobic and anaerobic conditions, respectively; *Streptococcus thermophilus* Rs, grown in a medium containing, per liter, 10 g of tryptone, 3 g of meat extract, 5 g of yeast extract (all from Difco Laboratories), 2 g of dipotassium monohydrogen phosphate, 40 ml of tomato juice, and 1 ml of Tween 80 (E. Merck AG, Darmstadt, Germany), pH 6.8, at 37°C; *Clostridium tyrobutyricum* BZ15, grown under anaerobic conditions in reconstituted *Clostridium* medium (E. Merck AG) at 30°C; and *L. lactis* subsp. *cremoris* BA3, grown in lactose-M17 medium at 30°C.

Antimicrobial activity was routinely determined by using an agar diffusion bioassay (28), with *M. flavus* or one of the other above-mentioned gram-positive bacteria as the indicator strain. Plates were prepared by adding a 0.1% inoculum of an exponentially growing culture of the indicator strain to 25 ml of growth medium solidified with 1.5% agar. After solidification in rectangular plates, wells (6.0 mm in diameter) were made with a sterile stainless-steel cork bore and filled with 40 µl of test solution. After incubation at 30°C (25 h for *M. flavus*), the radii of the halos were determined with a Nikon Profile Projector (model C; Nippon Kogaku, Tokyo, Japan). To determine the MICs of lantibiotics, exponentially

growing cultures were diluted 100-fold in media containing no (control) or different concentrations of purified lantibiotics and further incubated until control cultures were grown to saturation. The MIC was taken as the lowest concentration of lantibiotic that resulted in the absence of detectable growth. Cross-immunity was determined by following the growth of *L. lactis* NIZO R5 and NIZO 22186 in lactose-M17 containing different amounts of purified lantibiotics.

DNA manipulation, PCR, and DNA sequencing. *L. lactis* total DNA was isolated by lysing protoplasts (29) by the addition of 1% sodium dodecyl sulfate (SDS) and deproteinized by subsequent extractions with phenol and chloroform, which were followed by repeated ethanol precipitations (24). DNA was dissolved in 10 mM Tris HCl (pH 8.0)–1 mM EDTA, and approximately 100 ng was subjected to amplification by the polymerase chain reaction (PCR) (23) in a total volume of 50 µl containing 1 U of *Taq* polymerase (GIBCO-Bethesda Research Laboratories, Gaithersburg, Md.), 50 mM NaCl, 10 mM Tris HCl (pH 8.8), 2 mM MgCl₂, 10 µg of gelatin, 0.2 mM each deoxynucleoside triphosphate, 10 pmol of each primer, and 2.5 µl of stabilizer (1% N-1; GIBCO-Bethesda Research Laboratories) and covered with 100 µl of light mineral oil. The PCR amplifications were performed in 30 cycles, using a Thermocycler (Bio-Med, Amstelveen, The Netherlands); each cycle consisted of a denaturing step at 93°C for 2 min, a primer annealing step at 54°C for 1 min, and an extension step at 72°C for 1.5 min. The amplified DNA yielded a single band upon agarose gel electrophoresis (24) that was recovered with a Gene-Clean kit (Bio 101, La Jolla, Calif.). Sequencing of the thus purified DNA was performed by the dideoxy chain termination method (25), using the optimal conditions described by Casanova et al. (5), with the additional modification that all buffers and solutions contained 0.5% Nonidet P-40 (GIBCO-Bethesda Research Laboratories). Oligonucleotide primers used for PCR and for sequencing were 5'-CGCGAGCATAATAAACGGCT-3' and 5'-GGATAGTATCCATGTCTGAAC-3', which are complementary to regions 80 bp upstream and 29 bp downstream of the coding regions of the *nisA* and *nisZ* genes, respectively (18). Oligonucleotides were synthesized on a Cyclone DNA synthesizer (Biosearch, San Rafael, Calif.).

Purification and quantification of lantibiotics. Nisin variants were purified from culture supernatants of *L. lactis* NIZO R5 and NIZO 22186 grown for 16 h at 30°C as described before (19). Purification of the lantibiotics by using hydrophobic interaction chromatography on Fractogel TSK butyl 650-S (E. Merck AG), ultrafiltration, preparative reversed-phase high-performance liquid chromatography (HPLC), and freeze-drying was performed as described elsewhere (18). Purified lantibiotics were dissolved in 0.05% acetic acid, and absolute amounts were calculated from the A_{220} , using an extinction coefficient of 21.2 deduced from analyzing absorbance determinations and ¹H-nuclear magnetic resonance spectroscopy (21). In addition, the quantity and quality of the lantibiotic samples were determined by analytical reversed-phase HPLC performed on a Hi-Pore RP-318 column (250 by 4.6 mm; Bio-Rad Laboratories, Richmond, Calif.), using a linear gradient of 23 to 28% buffer B (90% aqueous acetonitrile, 0.07% trifluoroacetic acid) in buffer A (10% aqueous acetonitrile, 0.1% trifluoroacetic acid) for 50 min with a flow rate of 1 ml/min. Absorbance was monitored at 220 nm.

Gel electrophoresis and detection of nisin analogs. Tricine-SDS gel electrophoresis (26) of lantibiotics and low-molecular-weight standards (Bio-Rad Laboratories) on SDS-20%

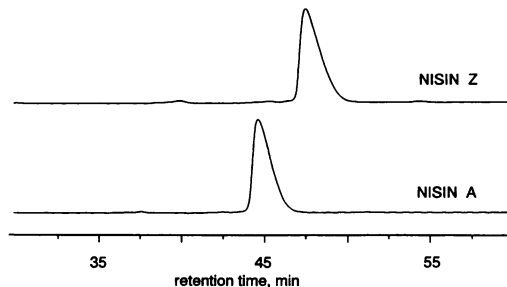


FIG. 1. Analytical reversed-phase HPLC of purified nisin A and nisin Z.

polyacrylamide gels was performed with unheated samples as described before (18).

RESULTS

Purification of nisin variants and differences in separation. Nisin Z produced by *L. lactis* NIZO 22186 (18) was purified and compared with purified nisin A isolated from *L. lactis* NIZO R5 known to contain the *nisA* gene (19). Analytical reversed-phase HPLC (Fig. 1) showed that both variants were >97% pure. In addition, this chromatogram showed that the nisin variants showed clear differences in elution times, with nisin Z eluting approximately 2 min later than nisin A. The purity of the lantibiotics was also evident from Tricine-SDS gel electrophoresis that showed single bands with identical apparent molecular weights of 3,500 (Fig. 2).

Differences in antimicrobial activities of nisin A and nisin Z. Initial experiments showed that the undiluted culture supernatant of NIZO 22186 resulted in much larger inhibition zones than that of NIZO R5 in a standard agar diffusion bioassay (28) with *M. flavus* as an indicator (data not shown). Therefore, the antimicrobial activities of the purified and standardized nisin variants were compared, using

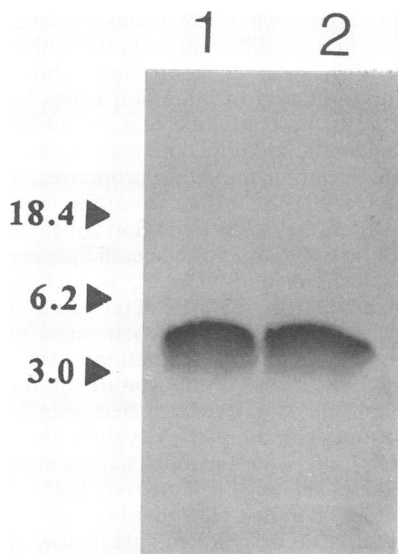


FIG. 2. SDS-polyacrylamide gel electrophoresis (26) of purified nisin A and nisin Z. Identical amounts (5 µg) of nisin Z (lane 1) and nisin A (lane 2) were applied, and the gels were stained with Coomassie blue. Molecular weight markers (10³) are indicated on the left.

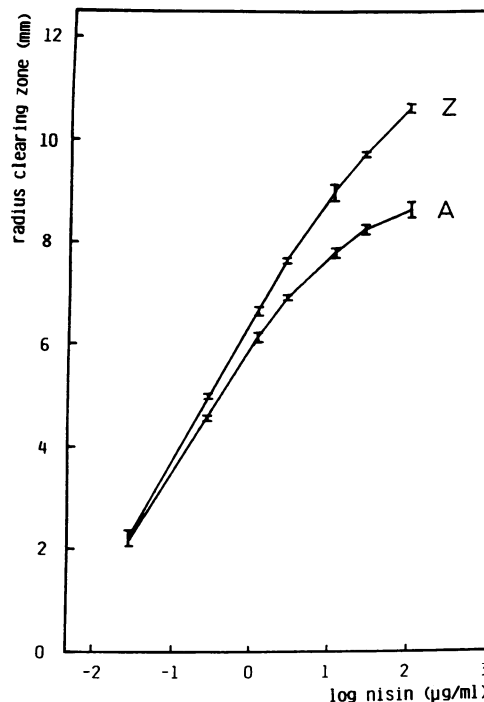


FIG. 3. Relation between the radius of the inhibition zone and the concentration of purified nisin Z (Z) or nisin A (A) in a standard agar diffusion assay with *M. flavus* as the indicator (28). Values have been derived from three independent experiments, and the standard error is indicated by the bars.

the same procedure. Significant differences between nisin A and nisin Z were obtained in dose-response curves that relate the radius of the inhibition zones to the concentration of added lantibiotic (Fig. 3). These differences include both the shape and the slope of the dose-response curves. With nisin Z there was a nearly linear relation between the radius of the inhibition zone and the logarithm of the concentration of lantibiotic up to approximately 10 µg/ml. In contrast, this linear relation was absent with nisin A above 1 µg/ml. The dose-response curves converged at a concentration of approximately 0.03 µg of lantibiotic per ml, where a similar inhibition zone was found.

Moreover, it appeared that the inhibition zones obtained with nisin Z were significantly larger than those obtained with nisin A at concentrations above 0.1 µg/ml, with a maximal difference at approximately 100 µg/ml. Careful inspection of the inhibition zones revealed that the boundaries of the halos obtained with nisin Z were less sharp than those of the halos obtained with nisin A (data not shown). This was most noticeable below 0.1 µg of lantibiotic per ml and was also observed when nisin Z was assayed with other nisin-sensitive bacteria (see below).

To study possible differences in specific antimicrobial activity, the MICs of both lantibiotics were determined in liquid cultures of *M. flavus*. The MICs appeared to be identical (0.015 µg/ml) for both nisin variants (Table 2) and were close to the concentrations that showed similar-sized inhibition zones in the agar diffusion test. These results demonstrate that there is no difference in specific activities of nisin A and nisin Z against *M. flavus*.

Spectrum of antimicrobial activity and cross-immunity. To compare the spectrum of antimicrobial activity of nisin Z

TABLE 2. Inhibition zones and MICs obtained for nisin A and nisin Z, using different nisin-sensitive indicator strains and nisin producers

Strain	Radius of inhibition zone (mm) ^a		MIC (µg/ml)	
	Nisin A	Nisin Z	Nisin A	Nisin Z
<i>M. flavus</i> DSM 1719	6.2	6.7	0.015	0.015
<i>S. thermophilus</i> Rs	4.0	4.5	0.030	0.030
<i>B. cereus</i> P7	2.4	3.2	0.3	0.3
<i>B. cereus</i> C5	4.2	5.3	0.1	0.1
<i>Listeria monocytogenes</i> 13	1.5	2.0	1	1
<i>Listeria monocytogenes</i> 669	3.0	3.5	0.2	0.2
<i>C. tyrobutyricum</i> BZ15	4.7	5.4	0.05	0.05
<i>L. lactis</i> subsp. <i>cremoris</i> BA3	3.6	4.2	0.05	0.05
<i>L. lactis</i> subsp. <i>lactis</i> NIZO R5	0	0	50	50
<i>L. lactis</i> subsp. <i>lactis</i> NIZO 22186	0	0	50	50

^a Values were obtained with identical concentrations (4 µg/ml) of lantibiotic in the agar diffusion test.

with that of nisin A, eight strains belonging to six known nisin-sensitive species were used as indicator organisms in agar diffusion tests and in liquid cultures to determine MICs (Table 2). In all cases, it appeared that the inhibition zones obtained with high concentrations of nisin Z were larger than those of nisin A. However, all strains that were sensitive to nisin A were also sensitive to nisin Z, and vice versa. The sizes of the inhibition zones varied considerably between the different indicator strains and were inversely related to the MICs. Interestingly, the MICs of nisin A were identical to those of nisin Z for all strains, indicating that there is no difference in the specific antimicrobial activities of the lantibiotic variants against these hosts.

When the lantibiotic producer strains NIZO R5 and NIZO 22186 were used in this assay, no inhibition zones were observed up to 100 µg of nisin A or nisin Z per ml, indicating that producer strains are immune to their own products (Table 2). In addition, these producer strains showed similar immunity to either of the lantibiotics in liquid medium (MIC of approximately 50 µg/ml), indicating cross-immunity to nisin A and nisin Z.

Distribution of *nisZ* and *nisA* genes in *L. lactis* strains. To determine whether NIZO 22186 was the only strain that produced nisin Z, the distribution of the *nisZ* gene was determined in 26 sucrose-fermenting and putatively nisin-producing strains of *L. lactis* from a variety of national and private collections as well as NIZO isolates (Table 1). Total DNA was isolated from these strains, and the structural nisin gene was amplified by PCR, resulting in a 0.35-kb DNA fragment. The presence of *nisA* or *nisZ* genes was determined by direct sequencing of the PCR product. The results (Table 1) show that *nisZ* genes are widespread in nature and are present in a frequency (14 of the 26) similar to that of the *nisA* genes in the tested strains. In all cases, a unique sequence ladder was obtained, indicating that a single *nis* gene species was present in the strains. In strain NCDO 2118, a small insertion of 16 bp was detected between positions 180 and 181 (numbering as in reference 18), which is located just downstream of the *nisZ* structural gene. This insertion consists of a tandem duplication of the octanucleotide AACCAAT present at positions 173 to 180. All other sequences were as reported for the *nisA* or *nisZ* genes, differing only in the nucleotide at position 148 (4, 18).

DISCUSSION

We describe the comparison and distribution of two naturally occurring nisin variants, nisin A and nisin Z (18). Nisin A was the first lantibiotic to be described (9, 17), and its structural gene (*nisA*) has been isolated from at least four different *L. lactis* strains (4, 7, 14, 19). Nisin Z is encoded by the *nisZ* gene and has recently been described in strain NIZO 22186 (18). The *nisA* and *nisZ* genes differ in a single nucleotide substitution at position 148 (18), resulting in the substitution of the histidine at position 27 in nisin A by an asparagine residue in nisin Z. It is shown here that this single substitution is widely distributed and results in different physicochemical, but not biological, properties of the nisin variants that are relevant to their application.

The most prominent property associated with nisin Z-producing *L. lactis* strains such as NIZO 22186 is the large size of the inhibition zones obtained in agar diffusion assays with undiluted culture supernatants, which is not observed to the same extent with known nisin A producers. This suggests a higher production level and/or a higher specific activity of nisin Z than nisin A. Careful comparison of purified and standardized nisin A and nisin Z preparations showed that, at concentrations above approximately 0.1 µg/ml, nisin Z produced significantly larger halos than the identical concentration of nisin A (Fig. 3). This observation appeared to be independent of the indicator organism (Table 2). In contrast, at a low concentration (0.03 µg/ml), no difference in the antimicrobial activities of the two variants could be detected (Fig. 3). This observation is in agreement with the identical MICs observed for nisin A and nisin Z with different sensitive bacteria and the cross-immunity of the producer strains (Table 2). These results indicate that there is no difference between the nisin variants in specific antimicrobial activity against the tested strains. A major implication is that the amino acid residue at position 27, being either asparagine or histidine, does not affect the biological activity of the variants and that the mode of action of nisin Z is comparable to that of nisin A, which has been studied extensively (8, 22).

The question arises, what determines the larger inhibition zones obtained in agar diffusion tests with nisin Z in comparison with nisin A at concentrations above the MICs (Table 2)? The formation of inhibition zones is affected by properties of both the lantibiotic (e.g., solubility, stability, aggregation behavior, and diffusion) and the indicator organism (e.g., growth rate, inactivation properties, and sensitivity). It is unlikely that the properties of the indicator organism are involved in the larger inhibition zones obtained with nisin Z, since the only lantibiotic-specific property, i.e., the sensitivity to nisin A and nisin Z, is identical for both variants as deduced from the MIC determinations (Table 2). This suggests that only the physicochemical properties of nisin Z are contributing to the formation of the large inhibition zones. On the basis of the nature of the His27Asn substitution in nisin Z, it is expected that nisin Z solubility is higher than nisin A solubility at pH values above pH 6 (the pK value of His) since the Asn side chain is more polar than the uncharged His side chain. However, both nisin variants have excellent and comparable solubility and stability at the concentration (up to 100 µg/ml) and pH (below 7) used in the bioassays (16, 21). This implies that a difference in the diffusion rate is the factor that contributes to the larger inhibition zones of nisin Z. This may be of practical significance since many products to which nisin is applied (12) are diffusion limited.

The marked differences between the shape and slope of the dose-response curves of nisin A and nisin Z (Fig. 3) have important implications for the accuracy of determining the nisin content of commercial and laboratory preparations. This is regularly done by comparing the inhibition zones of the unknown sample and a standard nisin A sample in an agar diffusion bioassay with *Micrococcus luteus* as the indicator (1, 28). The nisin content is subsequently expressed as international (Reading) units (1, 28). Since nisin Z shows much larger inhibition zones than nisin A at concentrations above approximately 0.1 µg/ml (Fig. 3), it is not possible to express nisin Z quantities in international units. This indicates that the conventional method is only applicable to samples known to contain only nisin A.

To study the distribution of nisin A and nisin Z producers, a large number of different *L. lactis* strains were analyzed at the gene level by PCR amplification of the *nis* gene and subsequent sequence analysis of the amplified DNA. This approach eliminated possible PCR errors that are often observed when amplified DNA fragments are first cloned prior to sequence analysis. *L. lactis* strains studied were selected for the capacity to ferment sucrose, a property often linked to nisin production, since the genes involved in these traits are physically linked and located on a conjugative transposon (19). Twenty-six strains, many of which were isolated from nondairy environments or as contaminants in milk, were found to contain the structural gene for nisin production, and in all cases a unique *nis* gene species was found (Table 1). About half (14 of 26) of the tested strains appeared to contain the *nisZ* gene sequence, indicating that nisin Z producers are widespread. This is supported by the finding that the *nisZ* gene is also present in *L. lactis* N8 (8a). These data imply that the first strain in which the *nisZ* gene was identified, NIZO 22186 (18), is not a rare mutant and suggest that the *nisA* and *nisZ* genes have diverged and subsequently have been disseminated in lactococcal strains. It is likely that the latter process is effected by conjugation since, analogous to nisin A production (19), nisin Z production also appears to be encoded by a conjugative transposon (designated Tn5278 [20]). As a consequence, it is not possible to establish a parent-progeny relationship between the *nisA* or *nisZ* genes, and hence both nisin A and nisin Z should be considered wild-type nisins. The screening approach described here also allowed the detection of new mutations. However, the sequence of all amplified *nis* genes was identical to that first described for either *nisA* (4) or *nisZ* (18), indicating that the silent mutation at position 168 (18) found in *L. lactis* 6F3 (14) is not widely present. Interestingly, a double duplication of an octanucleotide sequence was observed at the very end of the *nisZ* gene in strain NCDO 2118, but it did not affect the coding sequence.

The screening approach based on direct sequencing of PCR products that is used here in a search for natural nisin variants allows reproducible and rapid analysis of existing bacterial strains or new isolates. It is especially suitable for gene fragments or small genes, such as those encoding the production of lantibiotics or other bacteriocins. Complementary to protein engineering approaches, screening of naturally occurring strains, including nonproducers, may provide further insight into the structure, function, and evolution of nisin and other bacteriocins.

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