Developments in nisin research

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Nisin, produced by some strains of Lactococcus lactis, subsp. lactis was originally described in 1928 and is the most highly characterized bacteriocin produced by lactic acid bacteria. Nisin has been permitted as a food additive in the UK since the early 1960s and is currently an accepted food additive in at least 45 other countries. Technological advances have resulted in a tremendous increase in new information on nisin within the past decade. This review summarizes the recent developments in understanding the structure of nisin, the genetics of its production, and its mode of action.

Keywords: antimicrobial, bacteriocin, structure, genetics, immunity, resistance.

1 INTRODUCTION

Lactic acid bacteria produce a wide range of antimicrobial substances including bactercidal proteins or peptides known as bacteriocins (Klaenhammer, 1988). Nisin, for group N (streptococci) Inhibitory Substance (Mattick & Hirsch, 1947), is produced by some Lactococcus lactis subsp. lactis strains and is the most highly characterized bacteriocin produced by lactic acid bacteria. Nisin was initially described by Rogers (1928) as a substance that inhibits the growth of Lactobacillus bulgaricus and was suggested as the cause of slow acid development in cheese. Interest in nisin waned when it was shown that bacteriophage was the major cause of slow acid development in cheese manufacture (Whitehead, 1938). It was not until the 1940s that interest in nisin intensified, this time for its potential for use in food preservation.

Nisin is bactercidal against a broad range of gram-positive organisms including L. lactis subsp. lactis and subsp. cremoris, L. bulgaricus, Staphylococcus aureus, and Listeria monocytogenes and prevents the outgrowth of spores of many Clostridium and Bacillus spp. (Hurst, 1972; Harris et al., 1989). Although normally resistant to nisin, gram-negative organisms can be sensitized when the outer membrane is weakened in the presence of chelating agents (Stevens et al., 1991b), or by osmotic shock, or by the formation of cytoplasmic membrane vesicles (Kordel & Sahl, 1986). The fact that L. lactis strains are regarded as safe, coupled with the non-toxic nature of nisin, its sensitivity to α-chymotrypsin, and its heat stability at low pH has resulted in widespread use of nisin as an antimicrobial agent in the food industry. Nisin is permitted as a food additive in at least 46 countries, particularly for the inhibition of Clostridium spp. in cheese and canned foods (Hurst, 1981; Delves-Broughton, 1990).

The biology, chemistry, biosynthesis, and application of nisin to food have been frequently reviewed (Lipinska, 1977; Hurst, 1978, 1981; Eapen et al., 1983; Hurst, 1983; Rayman & Hurst, 1984; Delves-Broughton, 1990). The focus of the current review will be recent developments in understanding the structure of nisin, the genetics of its production, and its mode of action.

2 STRUCTURE

Nisin belongs to a group of antimicrobial peptides or bacteriocins known as lantibiotics which are produced by various gram-positive bacteria (Kellner...
Lantibiotics represent a class of closely related peptides that are effective against a broad range of gram-positive organisms (Sahl & Brandis, 1981; Kellner et al., 1988; Schnell et al., 1988). These bacteriocins have several unique features. They are small peptides (19 to 34 amino acids) and contain the unusual amino acids dehydroalanine (Dha), dehydrobutyrine (Dhb), lanthionine, and β-methyl-lanthionine. Dha and Dhb arise from dehydration of serine and threonine, respectively. Condensation of Dha or Dhb with cysteine generates thio-ether bonds and the amino acids lanthionine and β-methyl-lanthionine, respectively (Fig. 1).

The amino acid sequence of the precursors of nisin and some of the other lantibiotics is given in Table 1. In general, a high proportion of basic amino acids gives these peptides a net positive charge. Processed lantibiotic structures are shown in Fig. 2. Despite differences in amino acid sequence, the position of the first two lanthionine rings at the amino terminus of nisin, subtilin and epidermin is identical (Fig. 2). Pep 5 was initially believed to be structurally similar to nisin (Sahl et al., 1985). However, analysis of the amino and nucleic acid sequences of this bacteriocin revealed a structure with little apparent primary structural relationship to the other lantibiotics (Kaletta et al., 1989; Kellner et al., 1989).

Nisin is a peptide composed of 34 amino acids (3354 daltons) including one lanthionine, four β-methyl-lanthionine, one Dhb, and two Dha residues (Fig. 2). It does not absorb light at 280 nm since it contains no aromatic amino acids. Nisin can form dimers or oligomers which are thought to arise through a reaction between dehydroamino acids and amino groups of two or more nisin molecules (Liu & Hansen, 1990). Dha and Dhb are susceptible to modification by nucleophiles (hydroxyl groups or nucleophilic R groups) that are present at high pH; this may explain the instability and decreased solubility of nisin under basic conditions (Liu & Hansen, 1990). As pH increases, nisin solubility decreases from 57 mg ml⁻¹ at pH 2 to 0.25 mg ml⁻¹ at pH 8 to 12 (Lui & Hansen, 1990). Proton nuclear magnetic resonance (¹H NMR) analysis and computer simulation of nisin shows that it lacks regular secondary structure but exists in a rigid three-dimensional structure due to the constraints imposed by the five thio-ether rings (Chan et al., 1989b; Slijper et al., 1989). This is particularly evident at

![Fig. 1. Mechanism for the synthesis of unusual amino acids (dehydroalanine, dehydrobutyrine, lanthionine and β-methyl-lanthionine) found in nisin. Based on Ingram, 1970.](image-url)
the N-terminus where there are three adjoining lanthionine rings (residue 3 to 19) and a proline (Pro$_3$) residue within the second ring (Chan et al., 1989b; Palmer et al., 1989; Slijper et al., 1989).

Nisin preparations can be resolved into five polypeptides (nisins A to E) by counter-current distribution between solvents (Berridge et al., 1952). Relative proportions of the polypeptides vary with sample age and storage conditions. Nisins B to E are thought to be degradation products of nisin A. The amino acid sequence of nisins B to E was presumed to differ on the basis of activity, solvent migration, and sensitivity to nisin-inactivating enzyme (nisinase) (Jarvis & Farr, 1971).

Chemical degradation was used to determine the amino acid sequence of nisin A (nisin$_{1-34}$) (Fig. 1). This structure was confirmed by a combination of fast atom bombardment mass spectrometry (Barber et al., 1988), $^1$H NMR analysis (Chan et al., 1989b; Slijper et al., 1989), two dimensional NMR-techniques (Chan et al., 1989b), and total chemical synthesis (Fukase et al., 1988). $^1$H NMR analysis of nisin A degradation products shows that nisin$_{1-32}$ is nisin B and is as active as nisin$_{1-34}$ (Shiba et al., 1986; Chan et al., 1989a). The amino acid sequence of nisins C to E remains unknown. Nisin fragments, generated by treatment with cyanogen bromide, have varying antimicrobial activity. The specific activity of nisin$_{1-21}$ is reduced to about 10% of the activity of intact nisin (Shiba et al., 1986; Lui & Hansen, 1990). When the third ring of nisin$_{1-21}$ is opened at Met$_{17}$ or when this ring is removed by trypsin cleavage (nisin$_{1-12}$), activity is drastically reduced (Shiba et al., 1986). There are conflicting reports as to whether nisin$_{22-34}$ is inactive (Shiba et al., 1986), or has a 90% reduced activity (Liu & Hansen, 1990). Products of trypsin digestion of nisin (nisin$_{1-12}$ and nisin$_{13-20}$) are virtually inactive (Shiba et al., 1986) in contrast to early studies which showed no decrease in nisin activity upon treatment with trypsin (Jarvis & Mahoney, 1969). Further work is necessary to clarify which components of the nisin molecule are essential for activity.

Nisin$_{1-32}$ forms readily by cleavage of the

![Diagram](image-url)

Fig. 2. Structure of nisin and related lantibiotics. Dha, dehydroalanine; Dhb, dehydrobutyryne; Ala-S-Ala, lanthionine; Aba, $\alpha$-aminobutyric acid; Aba-S-Ala, $\beta$-methyllanthionine. All $\alpha$-carbon atoms of lanthionine and $\beta$-methyllanthionine are in the D-configuration. Adapted from Gross and Morell, 1971; Gross, 1975; Schnell et al., 1988.
<table>
<thead>
<tr>
<th>Lantibiotic</th>
<th>Amino acid sequence</th>
</tr>
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<tbody>
<tr>
<td>Nisin</td>
<td>I T S I S L C T P G C K T G A L M G C N M K T A T C H C S I H V S K</td>
</tr>
<tr>
<td>Subtilin</td>
<td>W K S E S L C T P G C V T G A L Q T C F L Q T L T C N C K I S K</td>
</tr>
<tr>
<td>Epidermin</td>
<td>I A S K F I C T P G C A K T G S F N S Y C C</td>
</tr>
<tr>
<td>Gallidermin</td>
<td>I A S K F L C T P G C A K T G S F N S Y C C</td>
</tr>
<tr>
<td>Pep 5</td>
<td>T A G P A I R A S V K Q C Q K T L K A T R L F T V S C K G K N G C K</td>
</tr>
</tbody>
</table>

* Adapted from Kellner et al., 1988.
† Gallidermin differs from epidermin in one amino acid.
Val$_{32}$-Dha$_{33}$ bond and is a result of the susceptibility of Dha$_{33}$ to hydrolysis under acidic conditions. Under autoclave conditions at pH 2 (mild acid) this bond is broken and nisin$_{1-34}$ is converted to nisin$_{1-32}$. Under strong acid conditions (hydrochloric acid in glacial acetic acid) and heat (50°C), (desÅl$_{33}$)nisin$_{1-32}$ is the major degradation product (Chan et al., 1989a). In (desÅl$_{33}$) nisin$_{1-32}$, the ring structure at Dha$_{33}$ is broken. The activity of this degradation product is reduced to less than 0.2% of the activity of nisin$_{1-34}$. It remains unclear as to whether or not residue five itself or the rigid ring structure surrounding this residue is important to activity. Early studies were incapable of detecting the break at amino acid five and, therefore, the incorrect assumption was made that loss of the last two amino acids (Dha$_{33}$-Lys$_{34}$) was sufficient to eliminate activity (Gross & Morrell, 1967; Jarvis & Farr, 1971).

In addition to chemically derived modifications of nisin A, nisin variations can also arise through changes in DNA sequence. Different _L. lactis_ subsp. _lactis_ strains produce nisin-like molecules with different activity spectra (Hirsch & Grinsted, 1951; Geis et al., 1983). Minor differences in amino acid sequence were proposed as an explanation for this phenomenon. _L. lactis_ subsp. _lactis_ strain NIZO 22186 produces a peptide (nisin Z) which is identical to nisin A except for a substitution of Asn for His at amino acid residue 27 (Mulders et al., 1991). This amino acid change is a result of a single nucleic acid substitution. A single amino acid substitution (Leu$_6$ for Ile$_6$) is also observed for two _Streptococcus epidermidus_ lantibiotics, epidermin and gallidermin (Table 1).

### 3 SYNTHESIS

Proteins containing amino acids which are not encoded by DNA can be produced either by pathways catalyzed by multienzyme complexes or through post-translational modification of a precursor peptide. Hurst (1966) suggested that the unusual amino acids in nisin were the result of post-translational modifications of a precursor nisin molecule. Actinomycin D, an inhibitor of mRNA synthesis, and inhibitors of protein synthesis such as chloramphenicol, puromycin, and tetracycline all suppress nisin synthesis. Ingram (1969, 1970) used radiolabeled amino acids to show for nisin that either serine or threonine combine with cysteine to give lanthionine and ß-methylanthionine, respectively. It was proposed that dehydration of serine and threonine residues occurred giving rise to dehydro forms, some of which could condense with neighbouring cysteine residues generating thio-ether cross-linkages (Ingram, 1970) (Fig. 1). Cloning and sequencing of the pronisin structural gene confirmed this hypothesis (Buchman et al., 1988; Kaletta & Entian, 1989; Dodd et al., 1990). Serine, threonine, and cysteine were located in the precise positions (Table 1) required to give a mature nisin molecule using the scheme outlined (Fig. 1).

Active nisin is initially detected in culture supernatants during late log or early stationary phases of growth. However, the nisin mRNA is expressed during logarithmic growth as well as in stationary phase (Buchman et al., 1988). The half-life of nisin mRNA is 7–10 min, much shorter than the exceptionally long 45 min half-life of subtilin mRNA (Banerjee & Hansen, 1988). Very little is known about regulation, production, or cellular location of nisin-processing enzymes.

### 4 GENETICS

The genetic basis for nisin production eluded researchers for many years. Kozak et al. (1974) were able to increase the selection of non-nisin-producing mutants in some strains under conditions which are known to enhance the curing of plasmid DNA (proflavine, ethidium bromide, elevated temperature). The ability to ferment lactose was also lost in some of these strains (Fuchs et al., 1975). Although not commonly observed, incompatibility between nisin production and plasmid-borne lactose-fermenting phenotypes has been reported (Steele & McKay, 1986).

Linkage of nisin production and sucrose-fermenting ability was noted in the 1950s (Hirsch & Grinsted, 1951). Of 18 nisin-producing _L. lactis_ subsp. _lactis_ strains tested, all were able to ferment sucrose, a trait normally considered variable in lactococci. LeBlanc et al. (1980) correlated loss of a 28-megadalton plasmid with loss of both nisin production and sucrose-fermenting ability in _L. lactis_ subsp. _lactis_ ATCC 11454. Although widely cited, the data to support this statement are not presented in the paper but are simply mentioned in the conclusions. Conjugal transfer of nisin production, nisin immunity, and sucrose-fermenting ability to nisin-negative _L. lactis_ subsp. _lactis_ strains was demonstrated with _L. lactis_ subsp. _lactis_ ATCC 11454 (NCFB496) (Gasson, 1984; Gonzalez
& Kunka, 1985; Steele & McKay, 1986) and seven other nisin-producing strains (Gasson, 1984). However, a specific plasmid linked to these phenotypes was not detected in the transconjugants. The genetic determinants for nisin production were recently shown to be chromosomally located in ten unique nisin-producing transconjugants (Horn et al., 1991), constructed in an earlier study (Gasson, 1984), and in ATCC 11454 (Steen et al., 1991). Sequence analysis of one of the transconjugants (conjugation of \( L. \) \( lactis \) subsp. \( lactis \) MG1614 and nisin-producing NCFB894) revealed that the nisin structural gene is located on a 70 kilobase-pair transposon, designated Tn5301 (Horn et al., 1991). (A transposon is a discrete DNA sequence capable of moving, independent of DNA homology, from one location to another.) In addition to sucrose fermentation, nisin immunity, conjugal transfer factors, \( N^8 \)-(carboxyethyl)ornithine synthase, and bacteriophage resistance determinants have been linked with nisin production (Donkersloot & Thompson, 1990; Gonzalez & Kunka, 1985; Murphy et al., 1988).

The genetic determinant for the nisin structural gene from three different \( L. \) \( lactis \) subsp. \( lactis \) strains has been cloned and sequenced (ATCC 11454, Buchman et al., 1988; 6F3, Kaletta & Entian, 1989; NCFB894, Dodd et al., 1990). The structural gene for the precursor nisin peptide has been designated spaN and nisA. The nucleic acid sequence of precursor nisin is identical in all three strains and encodes a 57-amino acid peptide, including a 23-residue leader region and a 34-residue structural region. The structural and leader region are cleaved at a characteristic proteolytic processing site (Pro\(_2\)-Arg\(_1\)-Ile\(_1\)) that is identical for nisin, epidermin, and galidermin (Schnell et al., 1988; Schnell et al., 1989). The leader peptide sequence of nisin shows a moderate degree of hydrophobicity which suggests a function such as directing the precursor peptide to a processing compartment perhaps in the membrane (Buchman et al., 1988).

Upstream of the precursor nisin gene (NCFB894 and NIZO R5 full sequence; ATCC 11454, partial sequence) lies an insertion element of 1241 or 1245 base pairs, IS904 (Buchman et al., 1988; Dodd et al., 1990; Rauch et al., 1990). An open reading frame encoding a putative transposase of 253 amino acids was identified. Although IS904 is located near the left end of Tn5301 (NCFB894), it is not believed to play a role in the transposition of Tn5301 (Horn et al., 1991).

The open reading frame immediately downstream of the nisin structural gene encodes for a protein of 851 amino acids (Steen et al., 1991). Computer analysis of the protein's secondary structure reveals numerous amphipathic helices which is an indication that the protein is membrane-associated. The protein lacks an amino terminus membrane insertion sequence and the authors suggest the protein is anchored on the cytoplasmic side of the membrane. The function of the protein is unknown, although it may play some role in nisin processing, export, or immunity.

The promoter for nisin has not been identified and Steen et al. (1991) propose that the nisin gene in ATCC 11454 is part of a polycistronic operon of greater than 8.5 kilobase pairs of which at least 4 kilobase pairs are upstream of the nisin gene. This is in contrast to other antibiotics such as subtilin and epidermin in which production is controlled by a monocistronic transcriptional unit (Banerjee & Hansen, 1988; Schnell et al., 1988). It is also in contrast to Horn et al. (1991) who place the left end of Tn5301 approximately 2 kilobase pairs upstream of the nisin structural gene. Continued upstream sequencing of this region of the \( L. \) \( lactis \) subsp. \( lactis \) ATCC 11454 chromosome should clarify this.

Nisin-producing strains are not typically used as components of mixed or multiple strain dairy starter cultures because of the nisin sensitivity of other starter culture strains. Conjugal transfer of nisin-producing ability to \( L. \) \( lactis \) subsp. \( cremoris \) has been demonstrated, expanding the opportunities to improve dairy starter cultures (Broadbent & Kondo, 1991). Conjugal transfer of nisin-producing ability to \( L. \) \( mesenteroides \) subsp. \( dextranicum \) was reported (Tsai & Sandine, 1987), but the transconjugant was highly unstable and nisin-producing ability was lost rapidly from the culture (Klaenhammer, 1991).

5 MODE OF ACTION

Nisin inhibits the outgrowth of spores and causes lysis of vegetative cells. It affects the post-germination stages of spore development; outgrowth is inhibited and vegetative cells are not formed (Campbell & Sniff, 1959). From the chemical structure of nisin and the potential reactivity of the dehydro groups it is suggested that the active site in spores is membrane sulphydryl groups present in newly germinated spores (Morris et al., 1984). The reactivity of dehydro residues in the
nisin molecule with mercaptans and complete loss of activity with loss of intact Dha\textsuperscript{3} further supports this hypothesis (Chan et al., 1989a; Liu & Hansen, 1990).

The primary target of nisin in vegetative cells is the cytoplasmic membrane, Ramseier (1960) noted strong adsorption of nisin to *Clostridium butyricum* vegetative cells. Nisin-treated cells leak ultraviolet-absorbing material and subsequently lyse. He concluded that nisin acts as a cationic surface-active detergent. Membrane disruption is now believed to be the result of incorporation of nisin into the membrane and subsequent ion channel or pore formation (Henning et al., 1986; Sahl et al., 1987; Kordel et al., 1989; Gao et al., 1991). Membrane potential is destroyed in sensitive gram-positive cells as a result of the efflux of K\textsuperscript{+}, amino acids, and ATP through the membrane pores (Ruhr & Sahl, 1985; Kordel & Sahl, 1986). Cytoplasmic membranes of gram-negative cells are sensitive but the effect is not observed unless the outer membrane is weakened (Kordel & Sahl, 1986; Blackburn et al., 1989; Stevens et al., 1991b). Destruction of the outer membrane can be achieved by osmotic shock, by formation of cytoplasmic membrane vesicles, or by procedures which affect the lipopolysaccharide component of the outer membrane (e.g., treatment with EDTA). Mutants of *Salmonella typhimurium* which have reduced membrane lipopolysaccharide are sensitive to nisin (Stevens et al., 1991a). A positive correlation is observed between sensitivity and degree of aberration in the lipopolysaccharide.

Nisin dissipates both the membrane potential and pH gradient in liposomes (artificial membrane vesicles) and, to a lesser extent, inhibits oxygen consumption by cytochrome c oxidase containing proteoliposomes (Gao et al., 1991). A membrane potential (negative inside) and/or a pH gradient (alkaline inside) is necessary for insertion and pore formation of nisin in the membrane. Phospholipid composition of the liposome affects the incorporation of nisin into the membrane and may account for differences in nisin sensitivity between bacterial species or strains.

Nisin and Pep 5 induce cellular autolysis in staphylococci (Bierbaum & Sahl, 1985, 1987). This may be another mechanism by which cellular lysis occurs. In addition to membrane disruption, there is some indication that peptidoglycan synthesis is partially inhibited by nisin (Linnnett & Strominger, 1973; Henning et al., 1986). Formation of a complex between nisin and the lipid intermediates of murein biosynthesis were shown to occur in vitro (Reisinger et al., 1980). High levels of nisin are necessary to inhibit murein biosynthesis, making it unlikely that this is the primary site of action. It is possible a nisin–murein complex is involved in initial nisin–cell interaction and/or in transport to the membrane.

6 Nisin Resistance and Immunity

In discussions regarding bacteriocins, the terms resistance and immunity are often used interchangeably. For the purposes of this review, immunity is defined as the self-protection mechanism of a producer strain to its own bacteriocin while resistance is defined as insensitivity of a non-producing strain. A major limitation for use of nisin as a food preservative has been natural variability in sensitivity among strains and occurrence of resistant strains. Naturally nisin-resistant bacteria have been isolated from bacon (Gibbs & Hurst, 1964), from cured and fermented meat products (Collins-Thompson et al., 1985), and from fermented vegetables (Harris et al., 1990). The development of acquired nisin resistance in the presence of sublethal nisin concentrations was observed for *Streptococcus agalactiae* (Hirsch, 1950), *Staphylococcus aureus* (Carlson & Bauer, 1957; Hossack et al., 1984), *Clostridium butyricum* (Ramseier, 1960), *L. monocytogenes* (Harris et al., 1991), and *Pediococcus pentosaceus* (Harris, unpublished data). The mechanism of nisin resistance has not been investigated and may differ from strain to strain. Strains which become resistant to nisin do not develop cross-resistance to antibiotics used in chemotherapy (Hossack et al., 1984).

In select cases, nisin resistance determinants have been linked to plasmid DNA in *L. lactis* subsp. *lactis* biavar diacetylactis (pNP40, McKay & Baldwin, 1984) and in *L. lactis* subsp. *lactis* (pTR1040, Klaenhammer & Sanozky, 1985; pSF01, von Wright et al., 1990). The nisin-resistant determinants from pNP40 (Froseth et al., 1988; Simon & Chopin, 1988) and pSF01 (von Wright et al., 1990) have been cloned and are expressed in strains of *L. lactis* subsp. *lactis*. Further subcloning and sequence analysis of the nisin-resistance gene from pNP40 revealed an open reading frame for a protein of 319 amino acids (Froseth & McKay, 1991). From the amino acid sequence, Froseth & McKay (1991) suggest a transmembrane location; however, the function of
the protein is, as yet, unknown. A DNA probe prepared from the pNP40 resistance gene did not hybridize to genomic digests of nisin-producing *L. lactis* subsp. *lactis* ATCC 11454, suggesting that the resistance and immunity factors are dissimilar. The immunity factor has not been identified although it would make sense that it is part of the putative nisin operon (Steen *et al.*, 1991). An open reading frame encoding an 851 amino acid protein downstream of the nisin structural gene has been determined and may function as an immunity protein, but this remains to be proven.

Another protein implicated in nisin resistance is nisinase, an enzyme which destroys nisin activity. Nisinase has been reported to be produced by *Lactobacillus plantarum* (Kooy, 1952), *L. lactis* subsp. *lactis* subsp. *cremoris, Enterococcus faecalis, Leuconostoc* sp. (Galesloot, 1956), *S. aureus* (Carlson & Bauer, 1957), *Streptococcus salivarius* subsp. *thermophilus* (Alifax & Chevalier, 1962) and several *Bacillus* spp. (Jarvis, 1967). Nisinase from *S. salivarius* subsp. *thermophilus* (Alifax & Chevalier, 1962) and *Bacillus cereus* (Jarvis & Farr, 1971) were partially purified but have not been fully characterized. Nisinase from *S. salivarius* subsp. *thermophilus* had no effect on subtilin while that of *B. cereus* inactivated nisins A, B, C and E and subtilin but not nisin D nor a variety of other peptide antibiotics. Further research to characterize nisinases and to determine their target site(s) is warranted.

**7 CONCLUSION**

Advances in chemical analysis and techniques in molecular biology have resulted in a rapid increase in understanding of the structure and genetics of nisin and related lantibiotics. Despite this, or perhaps because of it, many questions remain unanswered. Although the gene for prepronisin has been cloned, all of the genes necessary to produce a functional nisin molecule or any other lantibiotic have not been fully elucidated. Relatively little has been published on mechanisms of nisin resistance or immunity. Nisin-resistance determinants have application as selective markers for food-grade cloning vectors (Froseth & McKay, 1991; von Wright *et al.*, 1990) and their usefulness in starter cultures (nisin-resistant bacteria and nisin or nisin-producing strains) has been demonstrated (Harris *et al.*, 1990; Daeschel *et al.*, 1991). An understanding of the genetic and physiological basis for nisin tolerance would lead to further understanding of the mechanism of action of nisin. Finally, we need to evaluate the basic knowledge already gained in this area and apply it to the design of more effective antimicrobial systems for use as food preservatives, and more competitive starter cultures for food fermentations.

**REFERENCES**


