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APPLIED TECHNOLOGY

Developments in nisin research

Linda J. Harris,[a,b,c*] Henry P. Fleming[a,b‡] & Todd R. Klaenhammer[b,c,d]

[a]*Food Fermentation Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Departments of* [b]*Food Science and* [c]*Microbiology and* [d]*Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, North Carolina 27695–7624*

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 bactericidal proteins or peptides known as bacteriocins (Klaenhammer, 1988). Nisin, for group *N* (streptococci) *I*nhibitory *S*ubstance (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 bactericidal 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.*, 1991*b*), 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

* Present address: Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

‡ To whom correspondence should be addressed.

et al., 1988). 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-methyllanthionine. 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-methyllanthionine, 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-methyllanthionine, 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.*, 1989*b*; Slijper *et al.*, 1989). This is particularly evident at

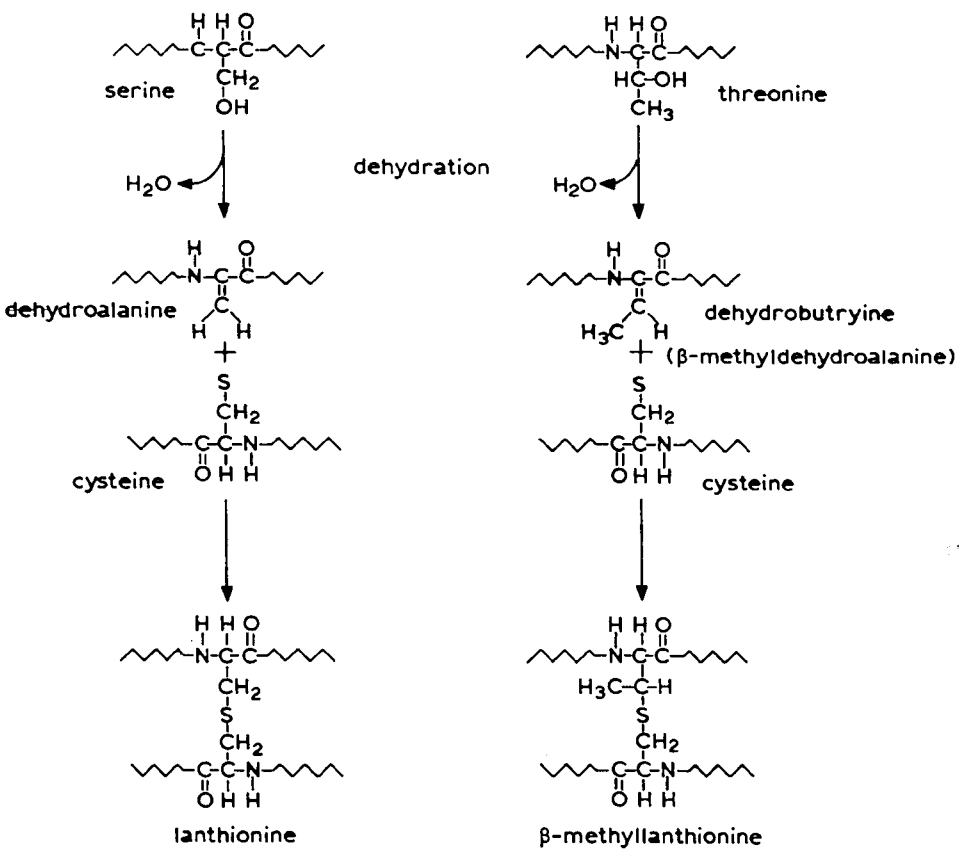

Fig. 1. Mechanism for the synthesis of unusual amino acids (dehydroalanine, dehydrobutyrine, lanthionine and β-methyllanthionine) found in nisin. Based on Ingram, 1970.

the *N*-terminus where there are three adjoining lanthionine rings (residue 3 to 19) and a proline (Pro_9) residue within the second ring (Chan *et al.*, 1989*b*; 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 spectroscopy (Barber *et al.*, 1988), ^1H NMR analysis (Chan *et al.*, 1989*b*; Slijper *et al.*, 1989), two dimensional NMR-techniques (Chan *et al.*, 1989*b*), and total chemical synthesis (Fukase *et al.*, 1988). ^1H 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.*, 1989*a*). 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

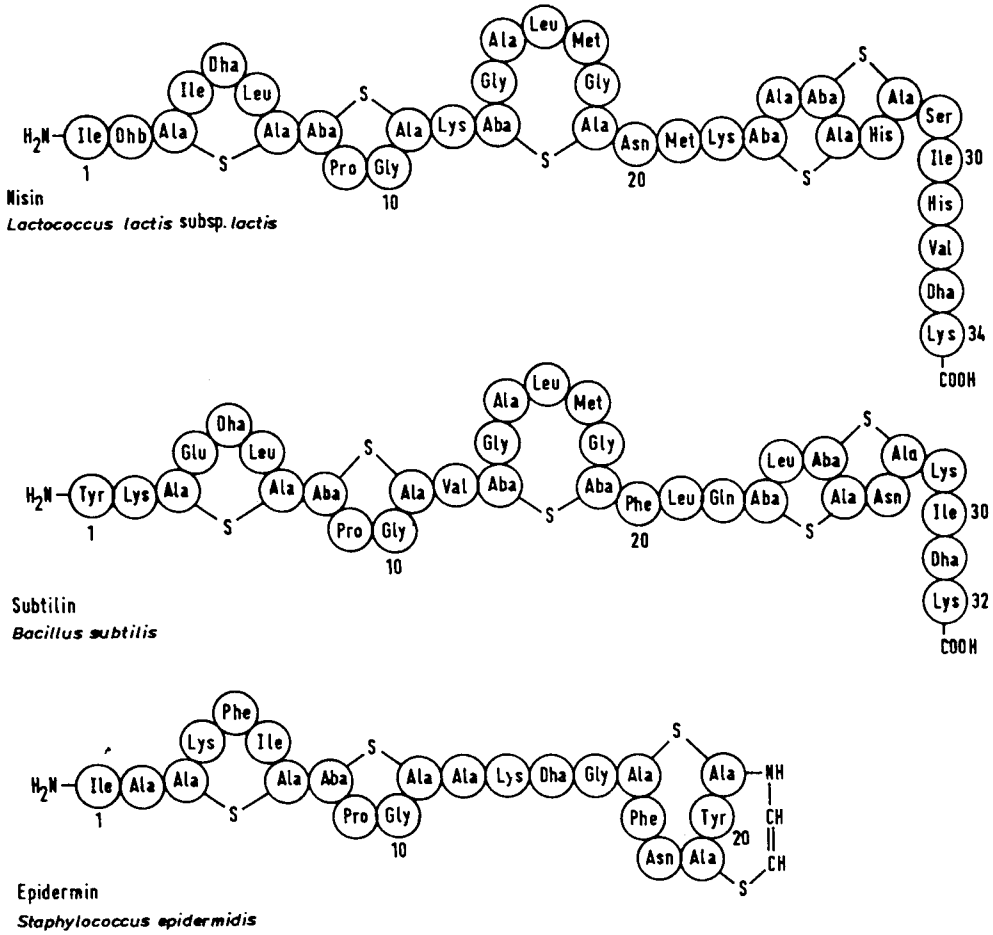

Fig. 2. Structure of nisin and related lantibiotics. Dha, dehydroalanine; Dhb, dehydrobutyrine; Ala-S-Ala, lanthionine; Aba, α-aminobutyric acid; Aba-S-Ala, β-methyllanthionine. All α-carbon atoms of lanthionine and β-methyllanthionine are in the D-configuration. Adapted from Gross and Morell, 1971; Gross, 1975; Schnell *et al.*, 1988.

Table 1. Peptide precursor sequences of selected lantibiotics*

Lantibiotic	Amino acid sequence
Nisin	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
Subtilin	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
Epidermin	I A S K F I C T P G C A K T G S F N S Y C C
Gallidermin‡	I A S K F L C T P G C A K T G S F N S Y C C
Pep 5	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

* 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), $(des\Delta Ala_5)nisin_{1-32}$ is the major degradation product (Chan *et al.*, 1989*a*). In $(des\Delta Ala_5)nisin_{1-32}$, the ring structure at Dha_5 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 β-methyllanthionine, 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 (proflavin, 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 Tn*5301* (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^5-(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 *spa*N and *nis*A. 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, IS*904* (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 IS*904* is located near the left end of Tn*5301* (NCFB894), it is not believed to play a role in the transposition of Tn*5301* (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 promotor 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 lantibiotics 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 Tn*5301* 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 *Leuconostoc 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[5] further supports this hypothesis (Chan *et al.*, 1989*a*; 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^+, 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.*, 1991*b*). 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.*, 1991*a*). 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 (Linnett & 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* biovar *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, L. 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.

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