Influence of the carbon source on nisin production in *Lactococcus lactis* subsp. *lactis* batch fermentations

LUC DE VUYST* and ERICK J. VANDAMME

Laboratory of General and Industrial Microbiology, Faculty of Agricultural Sciences, University of Gent, Coupure links 653, B-9000 Gent, Belgium

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Nisin production by *Lactococcus lactis* subsp. *lactis* NIZO 22186 was studied in batch fermentation using a complex medium. Nisin production showed primary metabolite kinetics: nisin biosynthesis took place during the active growth phase and completely stopped when cells entered the stationary phase. A stringent correlation could be observed between the expression of the prenisin gene (*nisA*) and the synthesis of the post-translationally enzymically modified and processed mature nisin peptide. Moreover, it seemed likely that nisin had a growth control function. A physiological link is proposed between sucrose fermentation capacity and nisin production ability. Carbon source regulation appears to be a major control mechanism for nisin production.

Introduction

Lactic acid bacteria produce a wide variety of antagonistic factors that include metabolic end-products such as lactic acid, antibiotic-like substances and antimicrobial proteins or bacteriocins (Klaenhammer, 1988; Lindgren & Dobrogosz, 1990). The antimicrobial activities of the industrially important lactic acid bacteria have long been known and traditionally applied, and these play an important role in food fermentations, food preservation and intestinal ecology (Gilliland, 1986, 1990). Among the antimicrobial proteins produced by lactic acid bacteria, nisin is the most studied (Hurst, 1981; Rayman & Hurst, 1984; Liu & Hansen, 1990).

The bioactive peptide nisin is synthesized by certain strains of *Lactococcus lactis* subsp. *lactis* (De Vuyst *et al.*, 1990) and is active against Gram-positive bacteria (Hurst, 1981). It has a molecular mass of 3500 Da and is composed of 34 amino acids, including three 2,3-unsaturated amino acid residues, one dehydroalanine and two 3-methyldehydroalanines or didehydrobutyrines, five thioether amino acids, one lanthionine and four 3-methylanthionines (Gross & Morell, 1971). Nisin is therefore a pentacyclic lantibiotic (Kaletta & Entian, 1989). The lantibiotics are a group of peptide antibiotics, containing 19–34 amino acids, which are characterized

by the occurrence of these thioether amino acids. They are produced from a ribosomally synthesized precursor peptide, which is subsequently post-translationally enzymically modified (Kellner *et al.*, 1988). The unusual amino acids should be responsible for the functionally important properties of the nisin molecule, i.e. thermostability and bactericidal action. Today, nisin is commercially produced exclusively by microbial fermentation (Vandamme, 1984) and is used in processed cheese and canned foods. However, it has many other potential applications as a biological food preservative (Hurst, 1981; Rayman & Hurst, 1984).

Information about the microbial production of the lantibiotics in batch fermentations is scarce (Sahl & Brandis, 1981; Kido *et al.*, 1983; Hörner *et al.*, 1989, 1990). Nisin batch fermentations have already been described (e.g. Hirsch, 1951; Hurst, 1966; Oberman & Jacubowska, 1968; Egorov *et al.*, 1971; Kozlova *et al.*, 1972; Lee & Kim, 1985) but so far there has been no systematic examination of nutritional parameters in relation to growth kinetics and lantibiotic metabolism in producing strains. The present study was undertaken to increase understanding of the physiology and metabolic control of nisin biosynthesis. Several attempts to increase nisin yields by genetic manipulation have been tried but were unsuccessful (Gasson, 1984; Tsai & Sandine, 1987). A better understanding of the nisin biosynthetic process and of its metabolic regulation seems to be a prerequisite for achieving improved nisin yields.

* Author for correspondence. Tel. (91) 646027; fax (91) 646245.

Abbreviation: CDW, cell dry weight.

Methods

Bacterial strains and media. The nisin-producing strain used in this study was *Lactococcus lactis* subsp. *lactis* NIZO 22186. It was stored at 4 °C in liquid yeast dextrose litmus milk (YDLM) medium consisting of (g l⁻¹): litmus milk (Oxoid), 100.0; yeast extract (Oxoid), 3.0; glucose, 10.0. The pH was adjusted to 6.80 before a threefold tyndallization at 100 °C for 30 min. A complex medium (CM-medium) was used for nisin production. It contained the following ingredients (l⁻¹): sucrose, 10.0 g; peptone (Oxoid), 10.0 g; yeast extract (Oxoid), 10.0 g; KH₂PO₄, 10.0 g; NaCl, 2.0 g; MgSO₄·7H₂O, 0.2 g. To prepare the inoculum, a similar medium was used which lacked peptone. In this publication, this modified medium will be referred to as inoculation medium. The initial pH of both media was adjusted to 6.80; the media were then autoclaved at 121 °C for 20 min.

Fermentation. *L. lactis* subsp. *lactis* NIZO 22186 precultures were grown for 5 h at 30 °C in 9 ml inoculation medium and subcultured for 10 h at 30 °C in 70 ml inoculation medium. A fresh culture of actively growing cells was always used as the inoculum; the inoculum size was always 1% (v/v). Fermentations were run in Erlenmeyer flasks (500 ml) containing 300 ml production medium and in a 6.2 l fermenter (New Brunswick Bioflo II) filled with 5 l of medium to ensure microaerophilic conditions. The Erlenmeyer flasks were incubated at 30 °C without agitation. The fermenter was operated at 30 °C without aeration; slow agitation (50 r.p.m.) was continuously provided to keep the fermentation broth homogeneous. The pH was measured (uncontrolled pH fermentations) or controlled continuously by automatic addition of 10 M-NaOH. Samples were withdrawn at regular intervals, and analysed for growth, nisin activity and residual sucrose concentration.

Growth determination. Growth was measured as optical density (OD) at 600 nm or by cell dry weight (CDW) determinations. The centrifuged cells (10000 g, 10 min) were washed twice with saline (0.85% NaCl).

Nisin activity determination. For estimating nisin activity, 1 ml samples were diluted with 9 ml 0.02 M-HCl. The tubes were then placed in a boiling-water bath for 5 min and then centrifuged (5000 g) for 10 min. The nisin activity of medium supernatant was determined by bioassay, which was performed by a quantitative agar diffusion method using *M. flavus* DSM 1790 (which is sensitive to nisin) (Tramer & Fowler, 1964). A standard curve (1–10 IU ml⁻¹) was plotted using a stock solution of 1000 IU nisin ml⁻¹. The latter was prepared by dissolving 100 mg of Nisaplin (Aplin & Barrett Ltd., Trowbridge, Wiltshire, UK) into 90 ml 0.02 M-HCl and 10 ml of the sterile fermentation medium used. Samples were diluted with 0.02 M-HCl. Each assay was performed in triplicate.

Sucrose concentration determination. Residual sucrose levels were determined enzymically using a Boehringer kit for sucrose determinations (Cat. No. 139041, Boehringer Mannheim).

RNA isolation and Northern blot analysis. *L. lactis* was incubated in M17 medium (Terzaghi & Sandine, 1975) supplemented with 5 g sucrose l⁻¹ as a carbon source, at 30 °C without aeration until an OD₆₀₀ of 0.8 was achieved. Cells were collected by centrifugation and resuspended in THMS buffer (30 mM-Tris, 3 mM-MgCl₂ in 25% (w/v) sucrose, pH 8.0) and subsequently digested with 50 µg lysozyme ml⁻¹ (Sigma) for 30–45 min at 37 °C. After centrifugation, the mixture was resuspended in 0.1 vols buffer I (0.5 M-sucrose, 20 mM-sodium acetate, 1 mM-EDTA, pH 5.5). Subsequently, 0.4 vols buffer II (20 mM-sodium acetate, 1 mM-EDTA, 0.5% SDS, pH 5.5) and 0.5 vols acid phenol (pH 5.5) were added, with occasional mixing. After incubation at 60 °C for 5 min, the water phase was separated and repeatedly extracted with acid phenol. The RNA was finally ethanol-precipitated. RNA

fractionation was performed on a denaturing formaldehyde gel, blotted onto a Gene Screen Plus nylon membrane (NEN, Du Pont) and hybridized. Hybridization was performed overnight at 42 °C with a natural prenisin structural gene – the *L. lactis* subsp. *lactis* NIZO R5 *nisA* gene – as a DNA probe, which had first been radioactively labelled by nick-translation. The blot was washed at 42 °C in 2 × SSC (1 × SSC is 0.15 M-NaCl plus 0.015 M-sodium citrate) supplemented with 0.1% SDS and subsequently developed by autoradiography at –80 °C.

Protein analysis. Proteins were analysed by Tricine-SDS-PAGE (Schagger & von Jagow, 1987), and silver-stained using BioRad reagents.

Results and Discussion

Dynamics of nisin formation in batch fermentations

Uncontrolled pH fermentations

The nisin production kinetics of *Lactococcus lactis* subsp. *lactis* NIZO 22186 were examined in the sucrose-based complex medium described. A batch fermentation profile of microbial growth and nisin production at uncontrolled pH is presented in Fig. 1. Exponential growth took place during a period of about 4–5 h at a maximum specific growth rate of 0.66 h⁻¹. Maximum cell density was reached after 8 h and amounted to 1.27 g CDW per litre of medium. Nisin production appeared to parallel that of biomass. Bactericidal nisin activity was already detectable after 4 h of growth. When about 60% of the biomass had been formed, there was a strong increase in nisin production. The highest nisin titre was reached at the end of the exponential phase, and corresponded with maximal biomass. Consequently, nisin was produced in the active growth phase. Nisin production completely stopped when cells entered the stationary phase. All these characteristics of nisin production clearly differentiate it from that of authentic secondary metabolites (Kleinkauf *et al.*, 1986; Martin & Liras, 1989). Almost all bacteriocins produced by lactic acid bacteria are produced during the active growth phase; further, the lantibiotics Pep 5 (Sahl & Brandis, 1981), ancovenin (Kido *et al.*, 1983), epidermin (Hörner *et al.*, 1989) and gallidermin (Hörner *et al.*, 1990) are also synthesized during the growth phase of the producer strain.

Despite rapid biomass formation and nisin production early in the culture, final high biomass and nisin levels could not be obtained, primarily because of an early sharp decrease in pH, owing to lactic acid accumulation. In the fermentation process with 10 g sucrose l⁻¹ as carbon source, the low pH presumably had an adverse effect on growth when about 60% of the sucrose had been converted to lactic acid. Moreover, during fermentations with increasing initial sucrose concentrations up to

Fig. 1. Batch fermentation profile of *Lactococcus lactis* subsp. *lactis* NIZO 22186 microbial growth and nisin production at uncontrolled pH. Cells were grown in a fermenter containing 5 l nisin production medium with 10 g sucrose l⁻¹ as carbon source (see Methods). Cell dry weight (g l⁻¹), ○; volumetric nisin titre (IU ml⁻¹), ●; residual sucrose concentration (g l⁻¹), □; pH, ■.

30 g l⁻¹, no more than 7.1 g sucrose l⁻¹ could be consumed. Even by increasing the initial sucrose concentration to 100 g l⁻¹, using Erlenmeyer flasks containing 300 ml production medium, maximum biomass averaged only about 0.85 g CDW per litre of medium, and the maximum nisin titre was approximately 2000 IU nisin ml⁻¹ irrespective of the addition of surplus sucrose. The maximal specific nisin production reached values of approximately 54 mg nisin (g CDW)⁻¹ [calculated for a pure nisin preparation with a specific activity of 40 × 10⁶ IU (g nisin)⁻¹].

The effect of pH on the growth of lactic acid bacteria is well known (Gilliland, 1986), but pH regulation of nisin biosynthesis must also be considered. pH regulation of the biosynthesis of antibiotics is not exceptional. For example, a pH-limiting effect was observed in *Bacillus brevis* gramicidin S production by Vandamme (1981), and Hörner *et al.* (1989, 1990) optimized epidermin and gallidermin production after an initial acidification of the culture medium.

Controlled pH fermentations

Fig. 2 represents the nisin batch fermentation profile under nutritional conditions as above, with 10 g sucrose l⁻¹ as a carbon source, but controlling the pH at 6.80. Neutralization of lactic acid by continuous addition of 10 M-NaOH increased the final biomass yield as well as

the nisin titre. With 10 g sucrose l⁻¹ as a carbon substrate, 2.34 g CDW per litre of medium could be obtained. However, growth had already ceased after 8 h of fermentation, mainly because no sucrose was left. The nisin titre increased almost proportionally with growth and reached its maximum, i.e. 1793 IU nisin (ml medium)⁻¹, at the end of the exponential growth phase, again corresponding with maximal biomass. The corresponding maximum specific nisin production was about 19 mg nisin (g CDW)⁻¹. After reaching their peak values, both the nisin and biomass levels dropped sharply. The high production rate of nisin during the microbial growth phase suggests that a continuous fermentation process could be considered for nisin production.

Expression of the prenisin gene (*nisA*)

Fermentation profiles indicated that nisin production shows primary metabolite kinetics. However, nisin production increased rapidly at the end of the exponential growth phase. To explain the observed nisin fermentation profile in detail, expression of the nisin precursor gene was studied by Northern blot analysis. *L. lactis* was therefore grown in M17 medium, supplemented with 5 g sucrose l⁻¹ as a carbon source. Growth was followed by measuring the OD₆₀₀. Samples were removed at different stages of growth (correspond-

Fig. 2. Batch fermentation profile of *Lactococcus lactis* subsp. *lactis* NIZO 22186 microbial growth and nisin production at controlled pH 6.80. Cells were grown in a fermenter containing 5 l nisin production medium with 10 g sucrose l⁻¹ as carbon source (see Methods). Cell dry weight (g l⁻¹), ○; volumetric nisin titre (IU ml⁻¹), ●; residual sucrose concentration (g l⁻¹), □.

ing to optical densities of 0.25, 0.50, 0.75 and 1.00) and centrifuged. The supernatant fluids were first assayed for nisin bioactivity and afterwards were lyophilized, TCA-precipitated and electrophoresed. The gel was silver-stained for protein, to reveal any nisin present. From the pelleted cells, total RNA was isolated, electrophoresed and blotted for Northern analysis, in which the labelled natural prenisin structural gene was used as a probe. These results are presented in Fig. 3. The growth curve shows that nisin activity could be detected early in the exponential growth phase, where a peptide band with a size of 3.5 kDa (corresponding to that of mature nisin) could also be seen. Because of the requirement for post-translational enzymic modification and processing of the nisin precursor, the prenisin gene would have to be transcribed before this event. Indeed, the Northern analysis revealed a hybridizing band with an apparent size of about 350 nucleotides that was already present in cells harvested in the early exponential growth phase, as well as in cells derived from the mid-exponential and late-exponential growth phases. Consequently, transcription of the structural nisin precursor gene and synthesis of prenisin had already started in the early exponential growth phase and continued until the stationary phase. Moreover, throughout the fermentation process, a good correlation existed between the

synthesis of this prenisin-mRNA (as revealed by Northern analysis), the occurrence of a mature nisin peptide band (as shown by the Tricine-SDS-PAGE) and the detection of nisin bioactivity in the fermentation broth (tested by bioassay). Such a well defined correlation exists, for instance, for the lantibiotic subtilin only in the stationary growth phase, where an increased induction of presubtilin-mRNA occurs (Banerjee & Hansen, 1988). These data indicate that the later increase in nisin bioactivity as compared to cellular growth can not be explained by a delayed expression of the nisin precursor gene, but would rather depend on the expression of the appropriate prenisin modifying or nisin generating enzymes.

Prolonged fermentations – functional role of nisin

After reaching peak values, the nisin levels and biomass decreased rapidly with a prolonged fermentation time, particularly in pH-controlled fermentations. Similar fermentation patterns were obtained for several other lactic acid bacteria bacteriocins, for instance lactacin DP1 production by *L. lactis* subsp. *lactis* (Piard *et al.*, 1988) and sakacin A production by *Lactobacillus sake* LB 706 (Schillinger & Lücke, 1989). The concentrations of other lantibiotics such as Pep 5 (Sahl & Brandis, 1981),

Fig. 3. Northern blot analysis of RNA isolated from different growth stages of *Lactococcus lactis* subsp. *lactis* NIZO 22186. (a) Growth (●) and nisin production (○) kinetics during batch fermentation at uncontrolled pH in M17 medium supplemented with 5 g sucrose l⁻¹. Growth was followed by measuring the OD₆₀₀. Cell-free culture supernatants were assayed for nisin bioactivity. Arrows indicate times at which samples were withdrawn for Northern blot and protein analysis. (b) Northern blot of RNA isolated from different growth stages. The time indications (1), (2) and (3) correspond to the arrows in (a). The blot was hybridized to the radioactively labelled natural

ancovenin (Kido *et al.*, 1983), epidermin (Hörner *et al.*, 1989) and gallidermin (Hörner *et al.*, 1990) have been shown to decrease as soon as the maximal antibiotic titres were reached. The decrease of the nisin titre after 8 h of fermentation implies that the fermentation process would need to be stopped by then to achieve a maximum specific nisin productivity.

Several hypotheses can be formulated to explain the sudden decrease of the biomass levels during nisin batch fermentation: an external bacteriophage attack during fermentation; induced lysis of a possible lysogenic producer strain by unknown factors; high intracellular accumulation of nisin and autolysis of the cells due to the exhaustion of the carbon source.

Kozak *et al.* (1973) reported that a high intracellular antibiotic concentration could also induce cell lysis. However, *L. lactis* subsp. *lactis* NIZO 22186 was immune to nisin up to an extracellular concentration of 10000 IU pure nisin (ml medium)⁻¹. Moreover, no intracellular nisin could be detected via Tricine-SDS-PAGE (unpublished data). Indeed, the mature active peptide should be secreted into the medium after processing (Kellner *et al.*, 1988). The fact that cell lysis always occurred at the same moment during different fermentations and the usual observation of the culture showing increased viscosity, are a possible indication of lysogeny. Also, UV irradiation of exponentially growing cells of the nisin producer strain *L. lactis* subsp. *lactis* NIZO 22186 induced lysis (data not shown). Indeed, most nisin producers seem to be lysogenic (Kozak *et al.*, 1973). Lysogeny together with the fact that several nisin producing *L. lactis* subsp. *lactis* strains possess a rather low proteinase activity (De Vuyst *et al.*, 1990), make it difficult to use these strains as starter cultures in cheese production on a large scale. However, a controlled cell lysis would be advantageous during cheese ripening because of the release of different peptidases and lipases.

Another possible explanation for the decrease in nisin titre is the release of non-specific proteolytic enzymes during cell lysis. Increased proteinase activity was observed towards the end of the fermentation cycle (data not shown) and the excretion of proteases during the stationary phase is common for dairy fermentations (Law, 1980; Law & Kolstad, 1983) and during fermentation of several other oligopeptide antibiotics. Continuous on-line adsorption of nisin onto an appropriate adsorber material during the fermentation process or the use of a membrane reactor could overcome proteolytic degradation. This has been experimentally and success-

prenisin structural gene (*nisA*) of *L. lactis* subsp. *lactis* NIZO R5. (c) Silver-stained protein profile of total protein in the supernatant fluids from samples removed at different times during growth, corresponding to arrows in (a). A band of 3.5 kDa, corresponding to the molecular mass of nisin, was present at all times nisin bioactivity was observed.

fully applied for epidermin and gallidermin (Hörner *et al.*, 1989, 1990).

It is also possible that the mature nisin peptide might have been destroyed by a nisinase, a specific nisin-destroying enzyme. Nisinases have already been isolated from *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus plantarum* and *Bacillus cereus* (Rayman & Hurst, 1984). Buchman *et al.* (1988) discovered an ORF upstream of the structural prenisin gene in the bacterial genome of the nisin producer *L. lactis* subsp. *lactis* ATCC 11454 that might be a candidate gene for a nisinase. However, it remains unclear why a nisin producer strain would produce a nisinase. This enzyme might be responsible for the high immunity of the producer strain against its own product. However, all these observations point to a possible regulatory function of nisin in the growth cycle of the nisin producing micro-organism, as postulated by Hurst (1978), although not all *L. lactis* subsp. *lactis* strains produce nisin. Whether other biologically inactive basic peptides in nisin non-producing *L. lactis* subsp. *lactis* strains (Hurst & Paterson, 1971), might have a similar growth control function to that suggested for producer strains, and what the actual role of the antibiotic activity would then be, remain unknown.

Effect of sucrose levels

Since nisin titre invariably seems to be proportionally related to biomass yield, especially under pH-controlled conditions, achieving a high biomass may be a prerequisite for high nisin production. Consequently, to improve the antibiotic yield further by generating higher cell densities, higher concentrations of the carbon source were tested in fermentations run at a controlled pH of 6.80. Using an initial concentration of sucrose of 20 g l⁻¹, the maximum biomass level was increased 1.5 times as compared with a fermentation with an initial sucrose concentration of 10 g l⁻¹. However, it did not prolong the active growth phase, and only increased the nisin activity by about 20%. A large increase in nisin production occurred only in the late exponential phase (Fig. 2). Higher concentrations of sucrose (greater than 20 g l⁻¹) yielded a higher biomass, but did not increase the nisin activity concomitantly (Table 1). The cell production amounted to 7.47 g CDW l⁻¹, whereas nisin production peaked at only 3267 IU nisin (ml medium)⁻¹, both at a level of 40 g sucrose l⁻¹. Because of this non-linear relationship between biomass yield and nisin production level, sucrose at 10 g l⁻¹ provided the highest specific nisin productivity [approximately 19 mg nisin (g CDW)⁻¹]. However, for high nisin yields, high specific nisin production is not sufficient. A critical amount of biomass seems to be a very important

Table 1. Influence of initial sucrose concentration on *Lactococcus lactis* subsp. *lactis* NIZO 22186 growth and nisin production in fermentations at controlled pH 6.80

Fermentations were run in a fermenter containing 5 l nisin production medium (see Methods). Samples were withdrawn at regular intervals. Results are maximum values obtained during fermentation.

Initial sucrose (g l ⁻¹)	Cell dry weight (CDW) (g l ⁻¹)	Volumetric nisin activity [IU nisin (ml medium) ⁻¹]		Y _{p/x} * [mg nisin (g CDW) ⁻¹]	Residual sucrose (g l ⁻¹)
		Maximum	After 24 h		
10.0	2.34	1793	617	19.1	0.0
20.0	3.40	2184	1620	16.0	0.0
30.0	4.53	2936	2286	16.2	1.2
40.0	7.47	3267	2671	10.9	0.2
50.0	5.79	2937	2714	12.7	2.8
70.0	4.74	1596	1300	8.4	18.7

* Y_{p/x} is the maximum nisin yield, calculated per gram CDW for a pure nisin preparation with a specific activity of 40 × 10⁶ IU (g nisin)⁻¹.

parameter. Up to a concentration of 40 g l⁻¹, all the sucrose initially supplied was completely consumed. Moreover, sucrose, although a disaccharide, is rapidly utilized by nisin-producing *L. lactis* subsp. *lactis* strains (see also Fig. 2), probably because they possess a very efficient phosphoenolpyruvate-dependent phosphotransferase system for its uptake, transport and metabolism (Thompson, 1988). With sucrose concentrations higher than 40 g l⁻¹, both biomass and nisin titres decreased. A limiting carbon source could not be responsible for this phenomenon or the observed cell lysis mentioned above, because relatively high levels of residual sugar still remained in the fermentation broth (Table 1). Generally, the higher the initial sucrose concentration, the greater the increase in nisin production at the end of the exponential phase and the lower the decrease in nisin titre during prolonged fermentation. Finally, a genetic linkage exists between sucrose fermentation capacity and nisin production ability (De Vuyst, 1990; De Vuyst *et al.*, 1990). A phenotypic correlation between sucrose fermentation and nisin production was first observed by Hirsch & Grinstead (1951) and has since been studied genetically by several investigators (Kozak *et al.*, 1974; Fuchs *et al.* 1975; Leblanc *et al.*, 1980; Gasson, 1984; Gonzalez & Kunka, 1985; Steele & McKay, 1986). In most cases, the sucrose and nisin genes were localized to a large plasmid, the so called sucrose-nisin-plasmid or pSN-plasmid. These conclusions were supported by successful curing and conjugation experiments. However, conflicting results have been obtained which suggest that nisin production is mediated by either

chromosomal or plasmid DNA (Davey & Pearce, 1982; Fuchs *et al.*, 1985). More recently, it has been shown that the structural genes for the nisin precursor (Buchman *et al.*, 1988; De Vuyst, 1990; Dodd *et al.*, 1990) and sucrose-6-P-hydrolase (P. J. M. Rauch & W. M. de Vos, unpublished results) are chromosomally encoded. Moreover, genes involved in nisin biosynthesis (Steen *et al.*, 1991) and sucrose fermentation (P. J. M. Rauch & W. M. de Vos, unpublished results) could form an operon, localized on a conjugative transposon of about 70 kb (Gasson, 1990). In any case, a genetic regulatory system or a common metabolic control system may be responsible for the linkage between sucrose fermentation capacity and nisin biosynthesis ability.

All the above results suggest carbon source regulation of nisin biosynthesis. Firstly a genetic regulatory system or a common metabolic control system for sucrose fermentation and nisin production capacity and dynamics may be responsible for the observed linkage between those metabolic traits. Secondly, because the small protein antibiotic nisin is synthesized via a chromosomally encoded precursor protein consisting of 57 amino acids (Buchman *et al.*, 1988; Dodd *et al.*, 1990), this precursor-nisin must be post-translationally modified by one or more enzymes (Kaletta & Entian, 1989). These modifications include dehydration of serines and threonines to double bond dehydroforms which then react with neighbouring cysteines to give lanthionine and 3-methyllanthionine (Ingram, 1970). Because Northern blot analysis revealed that the prenisin structural gene (*nisA*) is expressed early in the exponential growth phase, the large increase of nisin production towards the end of the active growth phase (see also Figs 1 and 2) could be the result of non-optimal nutritional control of microbial growth and subsequent delayed enzyme formation. According to the fermentation profiles of Figs 1 and 2, it seems possible that the formation of these prenisin-modifying enzymes or nisin-generating enzymes is carbon-source-regulated. These enzymes could be repressed during the early exponential growth phase and become derepressed later on. Hurst & Paterson (1971) had already postulated a maximal specific enzyme activity in the late exponential phase. After reaching their peak specific activities, the enzymes would rapidly disappear as the cells entered stationary growth phase. Consequently, derepression or activation of the processing enzymes by optimal nutritional and metabolic regulation could be responsible for higher nisin activity levels earlier in growth.

Nisin production clearly shows primary metabolite kinetics. According to the fermentation profiles presented here, it seems likely that nisin, or its precursor, has a growth control function. On the other hand, some results indicate that nisin production is regulated by the

carbon source. Expression of the prenisin structural gene occurs in the early exponential growth phase. Increased nisin production rate towards the end of the active growth phase could be due to delayed formation of the necessary prenisin-modifying enzymes. Whether the regulation is either genetically or enzymically mediated, and whether the processing enzymes are controlled by carbon catabolite repression, inhibition or inactivation is not yet known. Future investigations will be directed to the molecular mechanism of nisin biosynthesis, to the time-course of appearance and disappearance of the nisin-generating enzymes during batch fermentation, and to their regulation by different carbon sources.

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