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High nisin Z production by *Lactococcus lactis* UL719 in whey permeate with aeration

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The influence of controlled pH (5.0–6.5) and initial dissolved oxygen level (0–90% air saturation) on nisin Z production in a yeast extract/Tween 80-supplemented whey permeate (SWP) was examined during batch fermentations with citrate positive *Lactococcus lactis* subsp. *lactis* UL719. The total activity corresponding to the sum of soluble and cell-bound activities, as measured by a critical dilution method, was more than 50% lower at pH 5.0 than in the range 5.5–6.5, although the specific production decreased as pH increased. A maximum nisin Z activity of 8200 AU/ml (4100 IU/ml) was observed in the supernatant after 8 h of culture for pH ranging from 5.5 to 6.5. Prolonging the culture beyond 12 h decreased this activity at pH 6.0 and 6.5 but not at pH 5.5 or 5.0. A corresponding increase in cell-bound activity was probably due to adsorption of soluble bacteriocin to the cell wall. Aeration increased cell-bound and total activity to maximum values of 32 800 and 41 000 AU/ml (16 400 and 20 500 IU/ml), respectively, with an initial level of 60% air saturation after 24 h of incubation at pH 6.0. The specific production at 60% or 90% initial air saturation was eight-fold higher than at 0%.

Key words: Nisin Z, *Lactococcus lactis* subsp. *lactis*, citrate positive, whey permeate, aeration, pH.

Bacteriocins produced by lactic acid bacteria (LAB) are proteinaceous antimicrobial compounds with bactericidal or bacteriostatic activity against strains and species that are usually related to the producing strains (Tagg *et al*. 1976). In recent years, interest in bacteriocins has grown considerably because they are potentially useful for preventing the growth of spoilage and pathogenic bacteria such as *Listeria monocytogenes* and *Clostidium botulinum* (De Vuyst & Vandamme 1994).

Among several LAB bacteriocins, nisin A is the only one that is produced commercially by microbial fermentation (De Vuyst & Vandamme 1994). It is used as a food preservative in processed cheese and canned foods to prevent growth of clostridia and other pathogens (Delves-Broughton 1990). In view of the growing consumer demand for natural food additives, much applied research is currently focused on the possibility of producing large amounts of bacteriocins for this purpose. Conventional fermentation technology is limited, however, by low production rates and low product concentrations obtained in the fermentation broth (De Vuyst & Vandamme 1994).

Several studies have compared bacteriocin batch production by LAB strains on different complex media and have found that MRS and Elliker (ELB) broths were the best media for this production (Parente & Hill 1992; Daba *et al*. 1993). However, such media are too expensive for an economical production process. Commercial nisin A is currently produced by a pH-controlled batch-fermentation in a milk-based medium (De Vuyst & Vandamme 1994). Nisin can be recovered by the frothing technique (Halwley & Hall 1957). Recently, there has been increasing interest in producing bacteriocin from whey permeate since certain LAB strains can grow and produce appreciable amounts of bacteriocin in this low-cost medium (Daba *et al*. 1993; Liao *et al*. 1993; De Vuyst & Vandamme 1994) . The production of nisin in dairy media was studied by Huggenholtz & De Veer (1991); the highest nisin level was produced in milk (13 mg/l) followed by whey medium (8 mg/l).

Bacteriocins are generally extracellular products found in the supernatant solution. However, depending on the pH of the culture broth, some or all may be adsorbed to the

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producer cells (Lee & Kim 1985; Yang *et al*. 1992). Although the effect of pH has been demonstrated on the production of different bacteriocins (De Vuyst & Vandamme 1992; Daba *et al*. 1993; Parente *et al*. 1994), its impact on the distribution between solubilized and cell-bound forms has not been studied. Aeration largely increases the titre of lantibiotics, such as staphylococcal bacteriocin (Hörner *et al*. 1989; Tagg *et al*. 1976), but also decreases nisin A production by *Lactococcus lactis* (Hurst 1981).

Citrate positive *Lactococcus lactis* subsp. *lactis* (*Lc. lactis*) UL719 produces a nisin derivative (nisin Z), with a large inhibitory spectrum, particularly against *Listeria monocytogenes* and *Clostridium* sp. (Meghrous *et al*. 1997). Nisin Z differs from nisin A by a single amino acid substitution $His^{27} \rightarrow Asn$, which may account for the increased solubility of nisin Z at pH values above 6.0 (Mudlers *et al*. 1991). Among lactococcal strains, citrate positive *Lc. lactis* oxidizes NADH to NAD^+ by NADH oxidase (Bassit *et al*. 1993), so we investigated the possible stimulation of nisin Z production by aeration of the culture. The purpose of our investigation was therefore (i) to develop an inexpensive whey permeate basal medium to produce a high nisin Z titre using *Lc. lactis* UL 719, and (ii) to evaluate the effect of aeration and pH on producer strain growth, bacteriocin stability and soluble and cell-bound bacteriocin production.

Materials and Methods

Microorganisms

The citrate positive bacterium *Lc. lactis* UL719 which was isolated from raw milk cheese, and shown to produce nisin Z (Djae *et al*. 1995; Meghrous *et al*. 1997), was used for bacteriocin production. *Pediococcus acidilactici* UL5 (Daba *et al*. 1993) was used as the indicator microorganism for bacteriocin quantification due to its high sensitivity to the bacteriocin. Both strains were maintained in our culture collection as frozen stock cultures at −80 °C in MRS broth (de Man *et al*. 1960) obtained from Institut Rosell Inc. (Montréal, QC, Canada).

Growth Media

Whey permeate powder (edible dry whey permeate, Foremost Ingredient Group, Baraboo, WI) was rehydrated to a concentration of 6% (w/v), adjusted to a pH of 6.8 with 5 M NH_4OH and then centrifuged at 14 000 × *g* for 15 min at 4 °C to remove the precipitate. The supernatant solution was filter-sterilized using 50 mm bottle top 0.2 μm SFA filters (Nalgene Brand Products, Rochester, NY). Whey permeate was supplemented with sterile Tween 80 (0.1–0.4% w/v) or with sterile yeast extract (YE, 0.5–2% w/v) or with the same YE concentrations plus 0.1% Tween 80.

The effect of supplementation on nisin Z production was determined during batch fermentations with unregulated pH in 250 ml Erlenmeyer flasks containing 50 ml of medium, on a rotary shaker at 70 rev/min and 30 °C. Yeast extract/Tween 80-supplemented whey permeate medium was inoculated with 1% (v/v) stationary phase culture (8 h) of *Lc. lactis* UL719. The OD_{600} (optical density), the soluble and cell-bound bacteriocin titres were determined after 10 h and 24 h. Experiments were performed in duplicate.

A comparison of supplemented whey permeate (SWP containing 1% yeast extract and 0.1% Tween 80) with various complex media including MRS (Institut Rosell Inc., Montréal, QC, Canada), ELB (Elliker Lactic Broth, Difco, Detroit, MI) and BHI (Brain Heart Infusion, Difco) for bacteriocin production was also performed in duplicate following the same conditions and analyses as described above.

Fermentations

Fermentations for studying the effect of pH on nisin Z production in SWP (with 0.1% Tween 80 and 1% YE) were carried out in a 1.25 l-Bioflo (III) bioreactor (New Brunswick Scientific, Edison, NJ) at 30 °C with agitation at 70 rev/min. using a wide blade impeller, and 1.0 l of culture volume. The pH was controlled at 5.0, 5.5, 6.0 or 6.5 by adding 5 M NH_4OH. The inoculum was grown in 10 ml of the same medium in a 125-ml Erlenmeyer flask on a rotary shaker (70 rev/min, 30 °C). Samples were withdrawn from the fermentations at hourly intervals and analysed for cell concentration, soluble and cell-bound nisin Z activities, residual sugars and metabolic end product concentrations. The same experiment was carried out in MRS broth, which proved to be the best complex medium for bacteriocin production, with the pH controlled at 6.0, the optimal value determined in SWP.

Experiments for studying the effect of aeration on nisin Z production in SWP were conducted as described above, with a culture volume of 1.1 l and at pH 6.0. Filter-sterilized air was supplied to the fermenter beginning at 2.5 h after inoculation. Dissolved oxygen concentration (DOC) was measured using an autoclavable polarographic oxygen electrode (Phoenix Electrode, Houston, TX). Silicone antifoam 289 (Sigma Chemicals, St Louis, MO) was used to control foam formation. The different initial dissolved oxygen levels, 30, 60 and 90% air saturation, were obtained by keeping the air flow rate constant at 3.5 v/v/m (volume of air/volume of fermenter/min) and setting the agitation speed at 70, 150 and 300 rev/min, respectively. The experiments were duplicated and mean results are reported.

Analytical Methods

Biomass Measurements. Bacterial growth was estimated by optical density at 600 nm (OD_{600}) using a LKB diode array spectrophotometer (LKB, Cambridge, England) against a sterile medium reference. Viable cell counts were estimated by the pour plate technique. Each culture sample was treated with an Ultra-Turrax T25 mixer (Janne & Kunkel, Ika®-Labortechnik, Staufen, Germany) at 13 500 rev/min for 30 s and serial dilutions of bacterial suspensions were carried out in peptone water. Colony forming units (c.f.u./ml) were determined in triplicate after incubation for 48 h at 30 °C in MRS medium with 1.5% (w/v) agar (Difco). The maximum specific growth rate (μ_{max}) was obtained from the slope of the linear regression of ln(cell counts) against incubation time in the range 1–4 h, corresponding to the exponantial growth phase of the culture.

Bacteriocin Assay. Soluble and cell-bound nisin Z activities were determined by a critical dilution assay. Ten ml samples of each culture was centrifuged at 29 000 × *g* for 10 min at 4 °C. The supernatant were filtered though a 0.2-μm disposable sterile nylon filter (Cameo 25 N, MSI, Westboro, MA) to determine soluble bacteriocin activity. To determine cell-bound bacteriocin activity, the pellet was treated by the hot extraction method of White & Hurst (1968) modified as follows. The pellet was re-

suspended in 10 ml of 0.02 M HCl (pH 2.0), heated at 100 °C for 10 min, placed immediately in ice, and then centrifuged (29 000 × *g* for 10 min at 4 °C). Total activity was obtained by adding soluble and cell-bound activities.

Serial two-fold dilutions of bacteriocin preparations were performed in microcupules (disposible sterile multiple well plates, Cornings Glass Works, Corning, NY) that contained 125 μl of MRS broth, supplemented with 0.1% Tween 80. Supplementation of MRS with Tween 80 increased the sensitivity of the microtitration method by one cupule (two-fold factor). Each well was inoculated with 50 μl of an overnight culture of *Pediococcus acidilactici* UL5 (OD_{600} of 1.5), diluted 100-fold in MRS broth. After incubating the microtitre plates for 18 h at 30 °C, the activity of bacteriocin (AU/ml) was determined using the following formula: (1000/125)/D, where D is the highest dilution that prevents growth of the test organism after an incubation of 18 h. The bacteriocin assays were carried out in duplicate. The correspondence between arbitrary units (AU/ml) and international units (IU/ml) was determined by using Nisaplin (the commercial nisin preparation containing 2.5% nisin A, Aplin and Barrett Ltd, Beaminster, UK); in the critical dilution method described above, 2 AU corresponded to 1 IU (40 IU = 1 μg of pure nisin A). Reported activity data are means from replicated fermentations. The bacteriocin titres were highly reproducible for the repeated fermentations, and differed by no more than one well corresponding to a twofold dilution.

Determination of Sugars and Metabolic End Products. Sugars (lactose and glucose), organic acids (lactic acid, acetic acid), ethanol and acetoin were analyzed in duplicate by HPLC (Waters System, Milford, CT) equipped with an Ion 300 column and an IonGuard polymeric guard column (Interaction Chemicals Inc., Mountainview, CA). Lactic acid, acetic acid, ethanol and acetoin were detected by UV at 210 nm and sugars by refractive index. The mobile phase was 2.9 mM H_2SO_4.

Results

Effect of Whey Permeate Supplementation on Growth and Nisin Z Production

The growth of *Lc. lactis* UL719 in whey permeate without supplementation was minimal (OD_{600} = 0.435) with a maximum total activity of 272 AU/ml produced after 10 h of shake-flask culture followed by a decrease to 160 AU/ml after 24 h of incubation with a final pH of 4.1. Tween 80 supplementation (0.1–0.4%) of whey permeate produced no detectable increase in either cell growth or nisin Z production at any of the concentrations tested but did prevent the decrease in nisin Z titre that occurred in unsupplemented whey permeate after 24 h. A concentration of 0.1% Tween 80 was adopted for all subsequent experiments.

Yeast extract (YE) at 0.5–2% in whey permeate added with 0.1% Tween 80 approximately doubled both culture optical densities (OD_{600} = 0.85-0.97) and soluble nisin Z titre (512 AU/ml) compared with unsupplemented whey permeate. Cell-bound activity was always low (⩽16 AU/ml). In the absence of Tween 80, whey permeate supplemented with YE (1–2%) yielded large amounts of cell bound nisin Z activity (256 AU/ml), but a low soluble activity (32 AU/ml) after 24 h incubation.

Comparison of Supplemented Whey Permeate with Other Complex Media for Growth and Nisin Z Production

Production of nisin Z in whey permeate supplemented with 1% YE and 0.1% Tween 80 (SWP) was compared with that in the common media differing in nutrient content and buffering capacity. The highest growth was obtained in MRS broth supplemented with 0.1% Tween 80, followed by ELB, SWP and BHI, with respective optical densities at 10 h incubation of 1.14, 1.02, 0.91 and 0.87, which did not significantly change between 10 h and 24 h. Much greater differences among media were found for nisin Z production with titers of 1056, 576, 528 and 192 AU/ml after 10 h incubation in MRS, ELB, SWP and BHI, respectively.

Effect of pH on Cell Growth and Nisin Z Production in SWP

Batch fermentation profiles for cell growth and nisin Z production at different pH values are presented in Figures 1A–D. Biomass levels reached a maximum of 1.8×10^{10} (OD_{600} = 1.944) to 2.8×10^{10} c.f.u./ml (OD_{600} = 2.085) for pH varying from 5.5–6.5 after 8 h. At pH 5.0, the maximum biomass level was lower than for the other pH values, with 6.7×10^{9} c.f.u./ml (OD_{600} = 1.533) after 8 h. The maximum specific growth rate, μ_{max}, did not vary with pH in the range of 5.5–6.5, averaging 1.26 ± 0.03/h, but was lower at pH 5.0 (1.01 ± 0.02/h).

A maximum soluble nisin Z activity of approximately 8200 AU/ml was observed in the supernatant after 7 or 8 h of culture in the SWP for pH controlled at 5.5, 6.0 or 6.5. At pH 5.0, the soluble activity was 50% lower while biomass was about threefold lower. Prolonging the incubation beyond 12 h decreased the soluble nisin Z activity to 4096 and 2048 AU/ml at pH 6.0 and 6.5, respectively, with corresponding increases in cell-bound activity to 1024 and 4096 AU/ml, respectively (Figures 1C–D). However, both activities remained unchanged at pH 5.0 and pH 5.5. At this latter pH (5.5), the specific total activity was more than 50% greater than that at the higher pH values and comparable to that at pH 5.0, taking into account the low precision of the activity test. The production of soluble nisin Z also appeared to be coupled to cell growth, since it stopped when the culture entered the stationary phase. Total nisin Z activity, which corresponds to the sum of soluble and cell-bound activities, was maximum at approximately 8500 to 9000 AU/ml between 8 h and 12 h of culture for pH in the range 5.5–6.5, considering the low accuracy of the twofold microtitration method. Soluble activity represented a large part (more than 90%) of the total activity.

Lactose consumption was complete at pH 6.5 and pH 6.0, but not at pH 5.5 and pH 5 for which 78% and 23% of

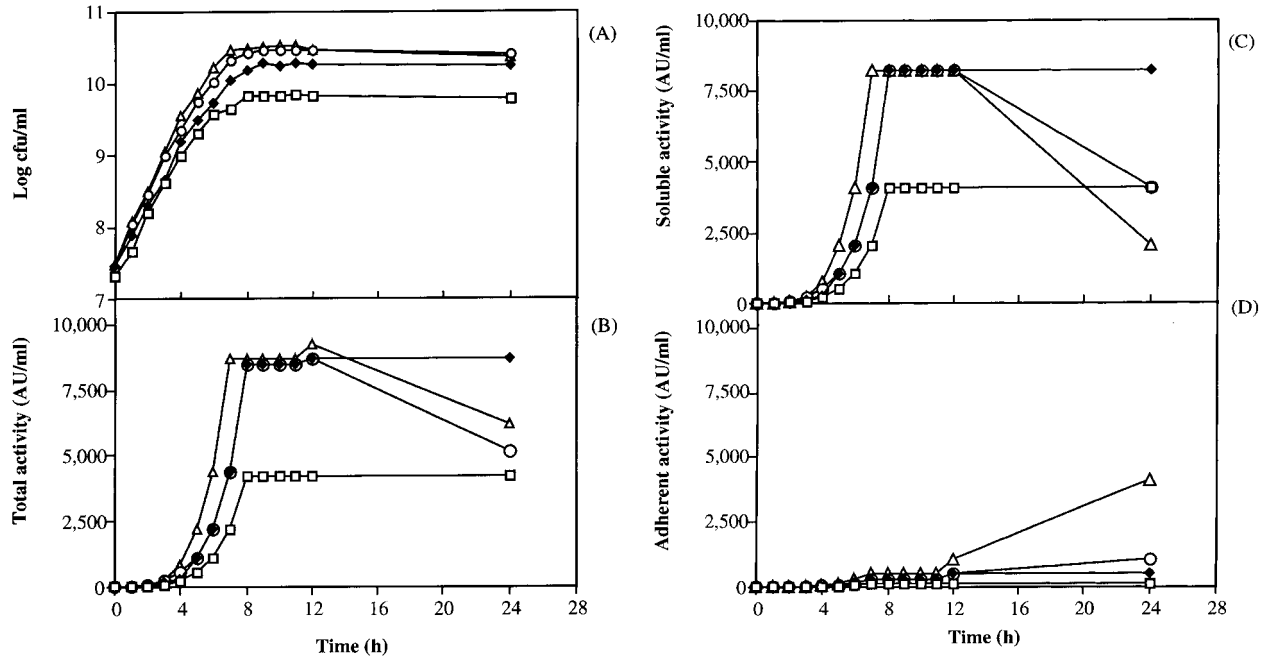

Figure 1. Time course of cell growth (a), total (b), soluble (c) and adherent (d) nisin Z production at different pH control set points during batch fermentation of SWP with *Lc. lactis* UL719: pH = 5.0 (□); pH = 5.5 (◆); pH = 6.0 (○); pH = 6.5 (△). Reported data are means calculated from two replicated fermentations. Bacteriocins titres differed by no more than one well and variation coefficients for plate counts did not exceed 12.5% for the repeated fermentations.

initial lactose (47.5 ± 2.0 g/l) was consumed at the end of incubation, respectively. Lactic acid concentration at the time corresponding to the maximal nisin Z production was four to six times greater at pH 5.5, 6.0 and 6.5 than at pH 5.0 with values of 34.8, 42.3 and 47.4 g/l, respectively, compared to 8.4 g/l.

Comparison of SWP and MRS Broth for Growth and Nisin Z Production with pH Controlled at 6.0

The time course for batch fermentations in SWP and MRS broth with pH controlled at 6.0, which was the optimum value observed in SWP, is shown in Figure 2. The cell concentration of 1.0×10^{10} c.f.u./ml in MRS broth was almost threefold lower than in SWP broth, but maximum specific growth rates were similar for both media (μ_{max} = 1.29 ± 0.03/h and 1.25 ± 0.03/h, respectively).

Similar maximum soluble nisin Z activities of approximately 8200 AU/ml were obtained for both media after 8 h of culture. Soluble titre in MRS broth fell rapidly after 11 h of culture from 8200 AU/ml to 4096 AU/ml at 12 h and finally to 512 AU/ml after 24 h. This effect was accompanied by an increase of smaller magnitude in cell-bound nisin Z activity from 512 AU/ml at 10 h to 2048 AU/ml at 12 h and finally to 4096 AU/ml after 24 h. At the same time, a drop in biomass level was also noted in MRS broth from 1.0×10^{10} to 2.1×10^{9} c.f.u./ml. A maximum total nisin Z activity of approximately 9000 AU/ml was obtained between 8 h and 11 h or 12 h of culture (Figure 2B), followed by a decrease in activity to 4608 AU/ml and 5120 AU/ml at 24 h for MRS and SWP, respectively.

Glucose was completely depleted in MRS broth at the time corresponding to the maxima for soluble nisin Z production and biomass, in contrast to SWP in which almost a third of the lactose had still not been utilized (data not shown). SWP contains nearly 50 g lactose/l, making the carbon source much more abundant, than in MRS broth (14 g glucose/l).

Effect of Aeration on cell Growth and Nisin Z Production in SWP

Cell growth and nisin Z production during fermentations at initial dissolved oxygen levels of 0, 30, 60, and 90% air saturation are presented in Figures 3A–D. Growth curves showed earlier decline phases and lower final counts for cultures aerated at 60% and 90% initial air saturation levels. Dissolved oxygen concentration (DO) dropped sharply after starting the aeration of the culture, to reach very low values for 30% and 60% initial air saturation levels (Figure 3A). However, with an initial level of 90% air saturation, the DO stayed above 20% throughout the course of the culture.

The maximum soluble nisin Z activity of 16 400 AU/ml in the supernatant was obtained at 9 h of culture at 30% and 60% initial air saturation (Figure 3C). Ninety per cent initial air saturation did not increase soluble

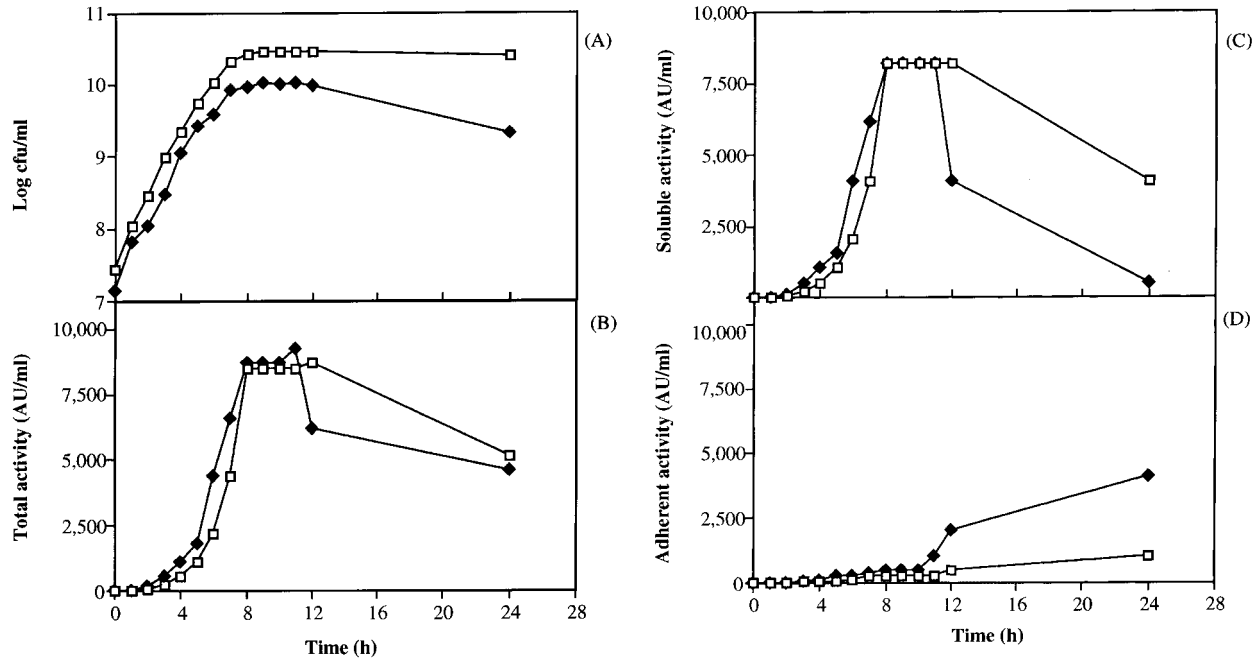

Figure 2. Cell growth (a), total (b), soluble (c) and adherent (d) nisin Z production during batch fermentation in SWP (□) and MRS (◆) broth with pH controlled at 6.0 with *Lc. lactis* UL719. Bacteriocins titres differed by no more than one well and variation coefficients for plate counts did not exceed 10.1% for the repeated fermentations.

nisin Z activity beyond that found without aeration. Beyond 12 h, soluble nisin Z activity decreased by 50% for all treatments. A more than proportional increase (two to four times the observed decrease) of cell-bound nisin Z activity was observed, reaching a maximum of 32 800 AU/ml after 24 h at 60% and 90% air saturation

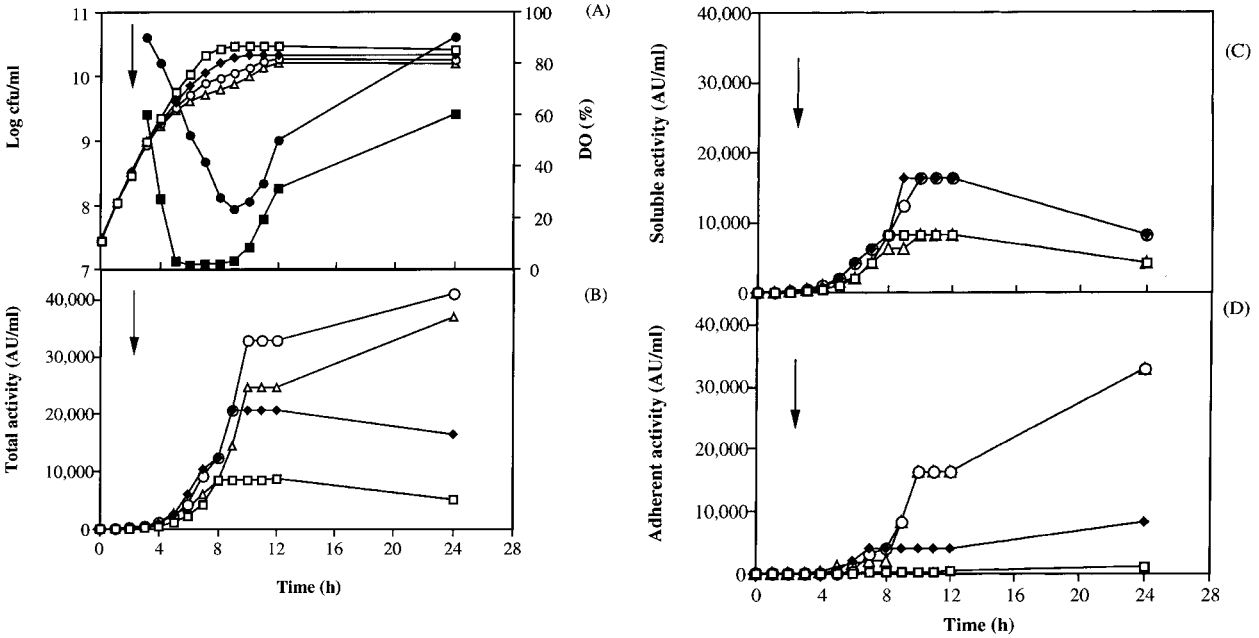

Figure 3. Profiles of cell growth and dissolved oxygen (DO) (a), total (b), soluble (c) and adherent (d) nisin Z production for various initial dissolved oxygen levels at controlled pH (6.0) during batch fermentation of SWP by *Lc. lactis* UL719. Initial dissolved oxygen level: 0% (□); 30% (◆); 60% (○); 90% (△). DO at 60% (■) and 90% (●) initial air saturation. The arrows indicate start of aeration of the culture, beginning 2.5 h after inoculation. Bacteriocins titres differed by no more than one well and variation coefficients for plate counts did not exceed 13.6% for the repeated fermentations.

(Figure 3D). Cell-bound nisin Z activity in the non-aerated culture increased at a much slower rate than that observed with aeration. After 24 h, cell-bound activity corresponded to approximately 20, 50, 80 and 89% of the total activity for 0, 30, 60 and 90% air saturation fermentations, respectively. Total nisin Z production at 24 h was greatest at 60% initial air saturation with 40 960 AU/ml followed by 90% initial air saturation with an activity of 36 900 AU/ml. Finally, specific nisin Z production at 60% (23.4 AU/10^7 c.f.u.) and at 90% (24.9 AU/10^7 c.f.u.) initial air saturation was more than twice that at 30% (10.6 AU/10^7 c.f.u.) and eight times the value at 0% (3.1 AU/10^7 c.f.u.). The same effect of aeration was observed when reporting specific activity in AU/unit OD.

Maximal total nisin Z activity corresponded to exhaustion of lactose for 60% and 90% initial air saturation, while at 30% air saturation only 65% of lactose was consumed at 9 h of culture when maximal activity occurred. The lactic acid production at the time corresponding to maximal total nisin Z production was similar at 0% (42.3 g/l) and at 60% and 90% initial air saturation (39 g/l), but was lower at 30% initial air saturation (28.3 g/l). Acetic acid, acetoin and ethanol production were also increased by aeration compared to non-aerated cultures (data not shown).

Discussion

Lc. lactis UL719 produced nisin Z in greater amounts in SWP containing 1% YE and 0.1% Tween 80 than in whey permeate alone mainly because of the presence of YE which increases cell production (Biswas *et al*. 1991; Daba *et al*. 1993). Most of the data reported on the effect of Tween 80 suggest a stimulatory effect on bacteriocin production (Biswas *et al*. 1991; Parente & Hill 1992; Daba *et al*. 1993), as measured in the supernatant alone. The present findings show that, when both soluble and adherent activity are considered, the apparent effect of Tween 80 may be explained by an increase in soluble bacteriocin production due to the release of cell-bound product rather than by stimulating bacteriocin production.

The greater nisin Z production obtained in MRS broth compared with the other media tested may be partly explained by the greater buffering capacity of this medium (Parente & Hill 1992) and the presence of glucose as the main carbon source (Reddy & Ranganathan 1983; Biswas *et al*. 1991). Most studies have reported that MRS is the best medium for bacteriocin production (Spelhaug & Harlander 1989; Daba *et al*. 1993), while others have shown that ELB is the best when MRS was not tested in the comparison (Geis *et al*. 1983; Reddy & Ranganathan 1983). This high production is probably due to the presence of YE or YE and bactopeptone in MRS and ELB media, respectively (Parente & Hill 1992; Reddy & Ranganathan 1983). The apparent absence of nisin Z inactivation for long incubation times in SWP in contrast to all other media is a factor worth considering. It has also been suggested that Ca^{2+}, which is present at high concentration in SWP, stimulates nisin Z production or displaces nisin Z that is adsorbed on the cell walls of producer cells (Matsusaki *et al*. 1996).

Coupling of nisin Z production with active growth (Figure 1) indicates a primary metabolite production which characterizes several bacteriocins or lantibiotics produced by LAB such as nisin A (De Vuyst & Vandamme 1992), pediocin UL5 (Daba *et al*. 1993) and lactococcin 140 (Parente *et al*. 1994). Soluble nisin Z titres decreased when extending the culture over 12 h at pH 6.0 with or without aeration and pH 6.5. This effect may be partly attributed to the readsorption of soluble nisin Z to the cell wall of the producer strain (Figures 1C–D and 3C–D) and/or to a decay in supernatant activity accompanied by continuous intracellular synthesis. The pH-dependent adsorption of the soluble bacteriocin to the cell walls is not surprising, due to the protein-like nature of the substance and given the known cation-exchanging properties of Gram-positive cell walls (Marquis *et al*. 1976; Yang *et al*. 1992). Nisin A, for example, has been shown to adhere to its producing bacterial cells in proportions as high as 95% at pH 6.8 in a complex medium supplemented with Na_2SO_4 (Lee & Kim 1985). Van't Hul & Gibbons (1996) showed that centrifugation apparently stripped producer cells of large quantities of loosely held nisin, even at pH 6.5, when no heat treatment was applied to culture samples. On the other hand, Yang *et al*. (1992) reported that nisin was adsorbed 100% to both producer and indicator cells at pH 6.5, and no activity was recovered in the supernatant after centrifugation at 17 000 × *g* for 5 min. This apparent discrepancy was tentatively explained by the denaturation of free/loosely held nisin during heat treatment at 70 °C for 25 min of culture samples to kill the cells and inactivate proteases (Van't Hul & Gibbons, 1996). In this study, no heat treatment was applied to culture samples before centrifugation and activity measurement in the supernatant. The high activities recovered in the culture supernatants obtained from fermentations with pH controlled at 5.0–6.5 are in agreement with data reported by Van't Hull & Gibbons (1996), eventually confirming the stripping effect of centrifugation on nisin loosely held to the producer cells.

Aeration was found to have a large stimulatory effect on nisin Z production by *Lc. lactis* UL719 in SWP. The decrease in soluble nisin Z production at 90% initial air saturation compared with 30% and 60% aeration may be

a result of foaming. Similar losses of soluble epidermin under vigorous aeration have been previously observed (Hörner *et al*. 1989). Both nisin Z and epidermin are surface-active polypeptides which when solubilized may aggravate foaming and incur activity losses. However, cell-bound nisin Z is apparently not affected by this phenomenon. Aeration has been found to be antagonistic to nisin A (Hurst 1981) and other bacteriocin production such as lactocin S (Mørtvedt-Abildgaard *et al*. 1995) and LIQ4 (Kühnen *et al*. 1985). On the other hand, aeration increased the specific production rate of amylovorin, a bacteriocin produced by *Lactobacillus amylovorus* (De Vuyst *et al*. 1996). Recently, Chinachoti *et al*. (1997) investigated the effects of aeration on nisin Z production by *Lc. lactis* IO-1. When aeration was increased during pH-controlled batch cultures, only a slight decrease of nisin Z production in the medium containing xylose was observed and the nisin Z titre did not decrease after the maximum was obtained during incubation. The authors hypothetized that aeration decreased the activities of degrading enzymes. An explanation of the high cell-bound nisin Z activity that resulted from aeration may require the elucidation of the biosynthetic pathway of bacteriocin production. Moreover, the distribution of nisin Z between soluble and cell-bound fractions is apparently largely influenced by medium composition (Tween 80) and aeration. Among lactococcal strains, citrate positive *Lc. lactis* can grow under aerobic conditions by reoxidizing NADH to NAD^+ by NADH-oxidase (Bassit *et al*. 1993); this activity could explain the O_2 consumption by *Lc. lactis* UL719 and the specific stimulation of nisin Z production by aeration, when started after 2.5 h incubation of the culture.

The maximum concentration of soluble nisin Z obtained in the pH-controlled non-aerated cultures (approximately 4100 IU/ml or 100 μg/ml) was comparable or higher than that generally obtained under the so-called optimal conditions for nisin A or nisin Z production (40–95 μg/ml or 1600–3800 IU/ml) (Rayman & Hurst 1984; De Vuyst & Vandamme 1992 & 1994; Matsusaki *et al*. 1996; Van't Hul & Gibbons 1997), taking into account the low precision of the activity test. Although other investigations have reported higher nisin production in industrial media, up to 6750 IU/ml (170 μg/ml), De Vuyst & Vandamme (1994) questioned the validity of these high nisin yields, which could be due to inaccurate bioassay procedures. In our study, aeration of the pH-controlled culture at 60% initial air saturation allowed total nisin Z production in SWP to increase to a very high value of approximately 20 000 IU/ml or 500 μg/ml. The high nisin Z production during culture of SWP with *Lc. lactis* UL 719 is a reflexion of the high biomass concentrations obtained, exceeding 10^{10} c.f.u./ml.

This study clearly shows that aeration is a major parameter to consider when optimizing nisin Z production in SWP by *Lc. lactis* UL 719 in addition to pH and medium ingredients, such as YE. The estimation of both soluble and cell-bound activities is recommended when studying the production of bacteriocin by fermentation since cell-bound activity may represent a considerable part of total activity depending on the conditions of the fermentation. High cell-bound nisin Z titres were obtained in the low cost, food grade SWP medium during aerated cultures. This characteristic may be advantageously exploited in the downstream processing of the bacteriocin and the production of natural food biopreservative ingredients with high antimicrobial activity.

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