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ORIGINAL ARTICLE

Culture medium of diluted skimmed milk for the production of nisin in batch cultivations

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Abstract Nisin is a promising alternative to chemical preservatives for use as a natural biopreservative in foods. This bacteriocin has also potential biomedical applications. Lactic acid bacteria are commonly cultivated in expensive standard complex media. We have evaluated the cell growth and nisin production of *Lactococcus lactis* in a low-cost natural medium consisting of diluted skimmed milk in a 2-L bioreactor. The assays were performed at 30°C for 56 h, at varying agitation speeds and airflow rates: (1) 200 rpm (no airflow, and airflow at 0.5, 1.0 and 2.0 L/min); (2) 100 rpm (no airflow, and airflow at 0.5 L/min). Nisin activity was evaluated using agar diffusion assays. The highest nisin concentration, 49.88 mg/L (3.3 log AU/mL or 1,995.29 AU/mL), was obtained at 16 h of culture, 200 rpm and no airflow ($k_L a = 5.29 \times 10^{-3}$). These results show that a cultivation medium composed of diluted skimmed milk supports cell growth to facilitate nisin biosynthesis.

Keywords *Lactococcus lactis* · Microaerophilic · Preservative · Lactic acid bacteria · Bacteriocin · Bioreactor

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Introduction

The bacteriocin nisin, first identified in 1928, is produced by certain strains of *Lactococcus lactis* subsp. *lactis* (Bhatti et al. 2004). Nisin has an antimicrobial activity spectrum against Gram-positive microorganisms, including bacterial spores, but shows little or no activity against Gram-negative bacteria, yeasts and fungi (Millette et al. 2004). However, Gram-negative cells can be sensitized with a chelating agent that alters the permeability of the outer membrane of these cells. Due to its antimicrobial properties, nisin was the first natural peptide granted a "generally recognized as safe (GRAS)" status in the USA, for use in processed cheese, and its application in various food products is allowed in several countries (Von Staszewski and Jagus 2008). The GRAS status of lactic acid bacteria (LAB) underlines their increasing use in traditional foods and in an expanding range of novel foods and products designed to have specific nutritional or other health-enhancing benefits (nutraceuticals, prebiotics, probiotics, etc) (Panesar et al. 2007). Important advances in this field include the use of nisin in the development of antimicrobial packaging (Guiga et al. 2009) and liposome encapsulation (Colas et al. 2007). Research has also revealed the potential of nisin as a therapeutic agent, such as in cattle mastitis (Cao et al. 2007), human ulcer caused by *Helicobacter pylori* (Flôres and Alegre 2001) and topical skin infections (Guerra and Castro 2002). It is also used for treating staphylococcal

mastitis in women (Fernández et al. 2008) and in cosmetic products (Liu et al. 2004) and as a vaginal contraceptive (Gupta et al. 2008). It worth noting that *L. lactis* has a promising application as an antigen delivery vehicle for the development of live mucosal vaccines as well as a cell factory for heterologous protein production with important biotechnological uses (Margolles et al. 2009; Nouaille et al. 2003).

The production of LAB and, more specifically, bacteriocin production is a very fastidious process due to the need for rich growth media containing nutrients, such as carbohydrates, nucleic acids, minerals, vitamins and, mainly, amino acids, proteins or protein hydrolysates (Vásquez and Murado 2008). In bench-scale studies, LAB are commonly cultivated in standard laboratory media [e.g. (de Man, Rogosa, Sharpe) MRS and M17 broth], but the use of these media is prohibitively expensive in large-scale production systems. In this latter case, high-cost complex culture media should be replaced by low-cost media to improve the commercial use of the biomolecule (Bernárdez et al. 2008; Guerra et al. 2007). Trinetta et al. (2008) formulated a culture media that was more economical than MRS broth, increasing sakacin A production from 180 to 480 AU/mL. Ostlie et al. (2005) evaluated the effect of temperature (20, 30, 37 and 45°C) on the growth and metabolism of six probiotic strains in an ultrahigh temperature (UHT) semi-skimmed milk medium supplemented with nutrients for 48 h. Their results showed that the stability of the number of viable cells was best at 30 and 37°C. Other researchers have focused on the use of industrial waste, such as milk whey (Arauz et al. 2008), fermented barley extract (Furuta et al. 2008) and mussel-processing wastes (Guerra et al. 2005), as substrates to generate high-valued bioproducts and also to contribute to decreasing environment pollution.

The development of applications for nisin in food and pharmaceutical industries is limited by the need for high-cost culture media, which eventually results in a high-cost product. Commercially available media are expensive, which has led researchers to search for cheaper formulations for bacteriocin production (Arauz et al. 2009; Vásquez et al. 2006). Bovine milk is a naturally complex medium with a high nutritive content, providing an excellent substrate for *L. lactis* growth and extracellular nisin release into the medium.

The objectives of our study were to evaluate the growth and nisin production of *L. lactis* subsp. *lactis* ATCC 11454 cells in a bioreactor containing UHT diluted skimmed milk as a low-cost alternative medium. The assays were performed at varying agitation and airflow rates in batch cultivations, namely, 100 rpm (no airflow rate, 0.5 L/min) and 200 rpm (no airflow rate, 0.5, 1.0 and 2.0 L/min).

Material and methods

Bacterial strains and media

The nisin-producing strain of *L. lactis* subsp. *lactis* ATCC 11454 and the nisin-bioindicator organism strain of *Lactobacillus sakei* ATCC 15521 were used in this study. Both microorganisms were maintained as frozen stock at −80°C in MRS broth (Difco, Detroit, MI) with 40% (v/v) glycerol. Distilled water was sterilized in bioreactor at 121°C for 15 min. UHT skimmed milk (Parmalat, São Paulo, Brazil) was heated at 111°C for 5 min in an autoclave. This medium was aseptically added to the distilled water inside the bioreactor [skimmed milk at 25% of its standard concentration (2.27 $g_{total\ solids}$)] . All chemicals were of analytical grade.

Batch cultures

The batch cultures were initiated by inoculating 160 mL of MRS broth with 300 μL (10^7 CFU/mL) of *L. lactis* cells in a 500-mL Erlenmeyer flask. The flask was then agitated on a rotary shaker at 100 rpm and 30±0.5°C for 36 h. A 10-mL aliquot of inoculum was removed for analysis, and remaining flask contents (150 mL) were used to inoculate a 2-L bioreactor (Bioflo 110; New Brunswick Co, NJ) containing 1.5 L diluted skimmed milk (pH 6.7).

Foams were controlled by adding 0.3 mL dimethylpolysiloxane (Sigma-Aldrich, Saint Louis, MO). The operating conditions were: (1) agitation at 100 rpm (airflow rate 0.5 L/min and no airflow) and (2) agitation at 200 rpm (airflow rate 0.5, 1.0 and 2.0 L/min, respectively, and no airflow), with the pH uncontrolled. The total cultivation time was 52 h at 30±0.5°C in order to observe variations in nisin activity associated with the various growth conditions. Samples were withdrawn at regular intervals to perform the analytical determinations. In order to verify possible contaminations, microscopic examinations were carried out using the Gram technique.

Analytical methods

Colony-forming units

Colony-forming units were determined by counting the number of colonies grown in MRS agar (Difco) at 30± 0.5°C for 24 h, relative to a sample previously diluted (10^{-1}–10^{-7}) sample in 0.85% saline solution (w/v).

Dissolved oxygen

Dissolved oxygen concentration was detected by an online polarographic probe (model InPro 6110/220; Mettler-Toledo, Alphaville-Barueri, Brazil) installed and sterilized

together with the vessel. The oxygen probe was calibrated by sparging the medium with air (dissolved oxygen tension 100%) and nitrogen (dissolved oxygen tension 0%); the 100% saturation value was based on air. The saturation point (100%) was calibrated 1 h before inoculation under established conditions (30±0.5°C, 200 rpm, 1.5 L/min airflow rate).

Mass transfer coefficient determination

The static gassing out method (Pirt 1975) was used to determine the mass transfer coefficient (k_La). The oxygen concentration of the solution was lowered by gassing the liquid out with nitrogen gas (oxygen free). Afterwards, aeration was initiated at a constant airflow rate, and the increase in dissolved oxygen tension was monitored using a polarographic probe.

pH monitoring

The pH of the medium during cultivations was measured by an online sterilizable electrode (model 405-DPAS-SC-K8S/225; Mettler-Toledo) and confirmed by using an external pH meter (Mettler Toledo model MPC0227).

Nisin activity determination

The activity of expressed nisin was evaluated using the agar diffusion method (Pongtharangkul and Demirci 2004). The cell suspension was first centrifuged at 13,200 *g* for 10 min at 10°C. The titer of nisin expressed and released in the culture media under the different conditions was then quantified and expressed in arbitrary units (AU/mL of medium) by agar diffusion (Penna et al. 2005) using *L. sakei* as the sensitive microorganism. *L. sakei* was grown in MRS broth with shaking (100 rpm at 30°C for 24 h).

The *L. sakei* bioassay agar plates consisted of 0.8% Bacto agar (Difco) and MRS broth. After autoclaving, the agar medium (100 mL) was cooled to 40°C and inoculated with 600 μL (OD_{660}=0.4) of the 24-h culture (50 mL) of the corresponding nisin-sensitive microorganism. A 20-mL aliquot of agar was aseptically poured into sterile petri dishes (100×15 mm) and, once the agar had solidified, 3-mm wells were cut out using a sterile pipe (total diameter 5 mm). From every sample, 50 μL of culture supernatant from the centrifuged *L. lactis* suspension was transferred into the wells on the surface of *L. sakei*-inoculated agar. The plates, not inverted, were incubated at 30°C for 24 h. The diameter of the growth inhibition zone was then measured in four directions, and the average diameter (±5 mm) of the halos was linked to the arbitrary activities (AU/mL) of nisin formed by the respective cultures. The results were compared to a commercial nisin standard curve.

For the standard curve, a stock solution of nisin was prepared by adding 1 g of commercial nisin (Nisin, Sigma, St. Louis, MO; standard at an activity of 10^6 AU, containing 25,000 μg nisin/g) to 10 mL of 0.02 M HCl. The relation between arbitrary units (AU/mL) and halo diameter (H, mm) was determined by concentrations of standard nisin (10^1–10^5 AU/mL) and the activity of nisin from the grown cells. Based on the calibration curves between arbitrary units per milliliter and international units per milliliter, 1.1±0.2 AU corresponded to 1.0 IU (40 IU= 1 μg of pure nisin A). The activity of nisin expressed in arbitrary units per milliliter was converted to milligrams per liters through the relation: nisin (mg/L)=(z × 0.025), where z = AU/mL and 0.025 is a conversion value related to 2.5% pure nisin.

Protein analysis

Residual protein levels were determinated using a bovine serum albumin standard according to the method of Lowry et al. (1951).

Lactose concentration

Lactose concentration of the cultivations was estimated enzymatically using β-galactosidase (Lactozym 3000L HP-G; Novozymes, Bagsvaerd, Denmark), and a glucose oxidase–peroxidase mixture (Laborlab, São Paulo, Brazil) (Sánchez-Manzanares et al. 1993).

Lactic acid quantification

Lactic acid production in batch cultures was determined by acid–base titration, using NaOH 0.01 M and an alcohol solution of 1% phenolphthalein (w/v) as indicator (Pereira et al. 2001).

Results and discussion

The main results of the batch cultures are summarized in Table 1. The cell growth profile, pH, nisin activity and lactic acid formation of six batch cultures are shown in Fig. 1.

Cell growth

All typical growth curves (Fig. 1) showed that nisin production in our system was closely related to growth (i.e. growth-associated), since its biosynthesis occurred during the exponential growth phase, showing primary

Table 1 Summary of experimental results obtained in the batch cultures

Assay number	Agitation (rpm)	Air flow (L/min)	Beginning of stationary phase (h)	μ_{max} (h^{-1})	X_{max} (CFU/mL)	P_{Nisin} (mg/L)	$Prod_x$ (CFU/mL/h)	$Prod_{Nisin}$ (mg/L/h)	Y	k_La (h^{-1})
1	100	–	14	0.12	$5.75.10^7$	12.43	$4.11.10^6$	0.89	4.88	$6.45.10^{-3}$
2	100	0.5	16	0.14	$1.45.10^8$	9.95	$9.06.10^6$	0.62	2.07	3.61
3	200	–	16	0.12	$9.55.10^7$	49.88	$5.97.10^6$	3.12	5.64	$5.29.10^{-3}$
5	200	0.5	16	0.13	$2.00.10^8$	15.77	$1.25.10^7$	0.99	3.16	5.96
6	200	1.0	16	0.17	$1.66.10^8$	17.70	$1.04.10^7$	1.11	2.88	9.82
7	200	2.0	16	0.16	$1.58.10^8$	17.70	$9.91.10^6$	1.11	2.33	4.33

μ_{max}, Maximum specific growth rate; X_{max}, maximum cell concentration; P_{Nisin}, maximum nisin concentration; $Prod_x$, cell productivity; $Prod_{Nisin}$, nisin productivity; Y, correlation between nisin activity (log AU/mL) and cell concentration (log CFU/mL); k_La, volumetric oxygen transfer coefficient. All of these parameters were determined at the beginning of stationary phase

metabolite kinetics. Therefore, the highest nisin titer was reached at the end of the exponential growth phase or at the beginning of the early-stationary phase and was related to the maximal biomass. This behavior has been observed by the authors in previous studies (Cabo et al. 2001; De Vuyst and Vandamme 1992; Lv et al. 2005). The absence of a

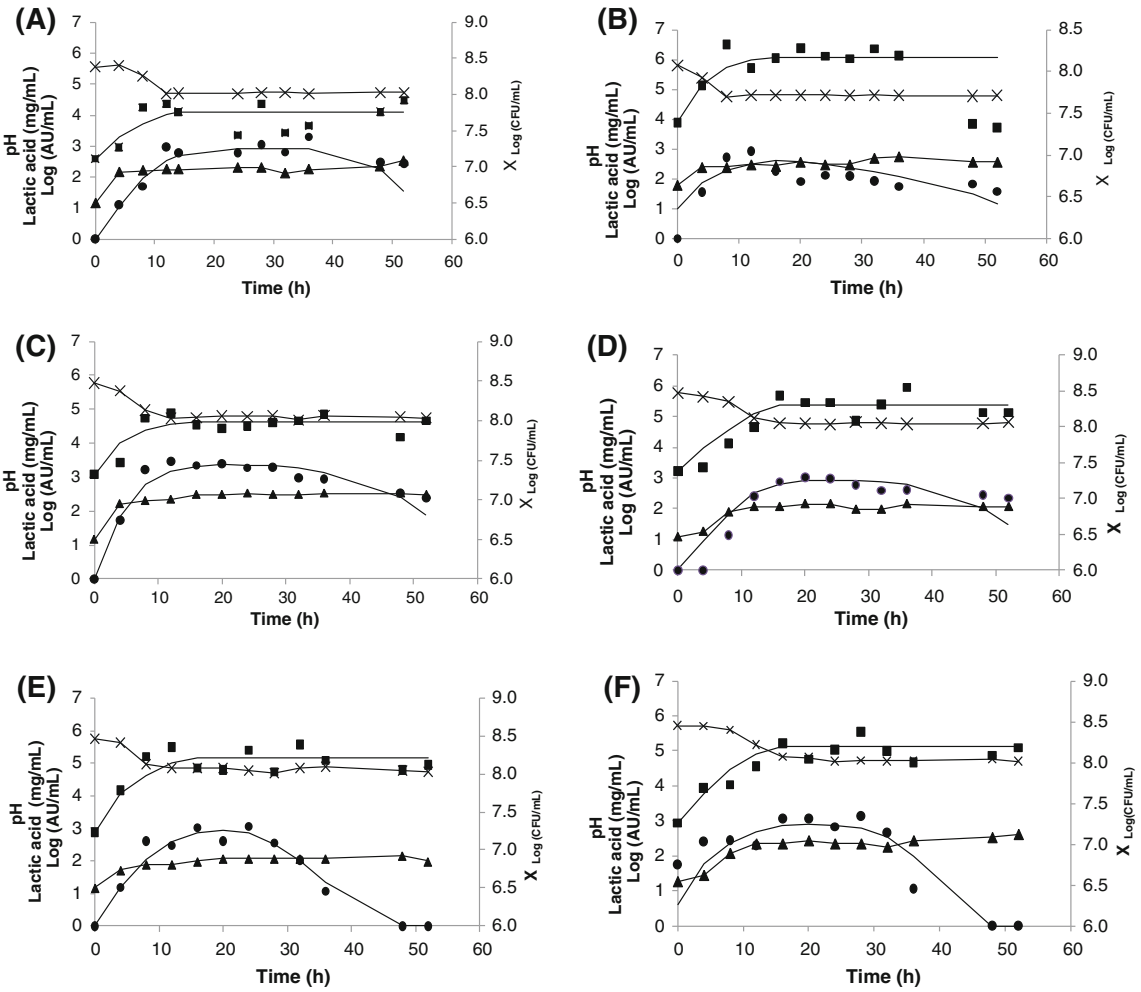

Fig. 1 Time profile of pH (*X*), nisin activity (log AU/mL; *solid circle*), microbial growth in colony-forming units (log CFU/mL; *solid square*), lactic acid (mg/mL; *solid triangle*) in batch cultivations of *Lactococcus lactis* in diluted skimmed milk in which the pH is uncontrolled, with varied agitation and airflow rates. Culture conditions were: **a** 100 rpm, no airflow rate; **b** 100 rpm, 0.5 L/min; **c** 200 rpm, no airflow rate; **d** 200 rpm, 0.5 L/min; **e** 200 rpm, 1 L/min; **f** 200 rpm, 2 L/min

"lag" phase suggests that inoculum cells were adapted to the medium. The results clearly indicate a short exponential growth phase time of approximately 16 h. Thereafter, all assays performed in the stationary phase obtained a maximum viable cell number of around 10^7–10^8 CFU/mL (X_{max}).

Nisin maximum concentration

As shown in Table 1 and Fig. 1c, the highest nisin activity was observed in assay 3 (200 rpm, no airflow) at 16 h, reaching a titer of 49.88 mg/L (1995.26 AU/mL), and it decreased after 32 h of culture. The best relation between cell growth and nisin production (5.64) was also observed in assay 3, indicating that the cells had adapted well to the medium and process conditions (Table 1). In contrast, the lowest maximum specific growth rate (0.12 h^{-1}) was obtained in assay 3. In this case, under microaerophilic conditions, the cells had only the residual oxygen contained inside of bioreactor at their disposal. López et al. (2007) studied enterocin EJ97 bacteriocin production in bovine milk (whole, half-skimmed, and skimmed) and obtained the highest titers (18 AU/mL or 11.25 mg/L) after 8 h of incubation in half-skimmed milk; this level is fourfold lower than that obtained in assay 3 (Table 1). These authors also verified that bacteriocin activity was much lower in whole milk than in half-skimmed or skimmed milk, suggesting that Ej97 may interact with milk fat. Jozala et al. (2007) reported a nisin concentration of 31.38 mg/L (1255.16 AU/mL) during an interval of 20–30 hours in batch culture using diluted skimmed milk with 2.27 $g_{total\ solids}$. Therefore, the levels obtained in our study (Table 1) are approximately 60% higher than those obtained by these authors. The same authors utilized MRS broth (Difco) as a preculture in *L. lactis* cultures at 36 h and obtained nisin concentrations of 48 mg/L or 1,920.7 AU/mL, which is similar to the levels obtained in our study at 16 h of cultivation on the low-cost medium.

The lowest value of produced nisin was obtained in assays with low agitation (100 rpm) and aeration of 0.5 L/min (K_La=3.61 h^{-1}) and without airflow (K_La=6.45×10^{-3} h^{-1}), corresponding to maximum nisin concentration of 9.95 mg/L (398.11 AU/mL) and 12.53 mg/L (501.19 AU/mL), respectively. This fact can be related to low medium homogenization.

The purposes of aeration and agitation in bioreactors are (1) to supply microorganisms with oxygen and (2) to mix the fermentation broth in such a way that a uniform suspension of microbes is achieved and the mass-transfer rate of the metabolic product accelerated (Aiba et al. 1973). Oxygen transfer is often the rate-limiting step in the aerobic bioprocess due to the low solubility of oxygen in the medium. The dissolved oxygen concentration in a suspension of aerobic microorganisms depends on the rate of gas–liquid oxygen transfer at which oxygen is transported into the cells (where it is consumed) and on the microorganism oxygen uptake rate for growth, maintenance and production. The correct measurement and/or prediction of the volumetric mass transfer coefficient (k_La) is a crucial step in the design, operation and scale-up of bioreactors (Garcia-Ochoa and Gomez 2009).

LAB species are classified in the literature as either anaerobic or microaerophilic (Jensen et al. 2001) because these species do not have catalase, which is widely distributed among aerobic bacteria. Oxygen toxicity in a cell is attributed to the activity of reactive oxygen species (hydrogen peroxide and hydroxyl radicals) that attack proteins, lipids and nucleic acids. However, a cell's aerotolerance is related to its ability to induce superoxide dismutase and NADH oxidase (Jiang and Bommarius 2004). The effects of oxygen on *L. lactis*, which has a fermentative metabolism that can use different sugars to produce mainly L-(+)-lactic acid, have been examined. Oxygenation of cultivations results in an altered redox state and greater NADH oxidase activity; as a consequence, sugar fermentation is shifted toward mixed fermentation, and acetic acid, formic acid, CO_2 ethanol and lactic acid are produced (Duwat et al. 2001). Despite these changes, *L. lactis* has been studied under both anaerobic (Lv et al. 2005) and aerobic conditions (Cabo et al. 2001; Liu et al. 2006). Under aerobic conditions, the addition of exogenous catalase (Duwat et al. 1995) or hemin (Berlec et al. 2008) was found to improve the survival of *L. lactis* cells exposed to oxygen. Additionally, aeration conditions for the maximum production of both biomass and bacteriocins—not always coincident events—can differ between species (Vásquez et al. 2004). It should be mentioned that, in terms of biopharmaceutical and vaccine production, aerobic processes usually are safer and more economical than anaerobic processes.

In order to extend our knowledge of the aerobic behavior of *L. lactis* cell growth and its concomitant nisin release into the media, we designed two assays in which the cells were shaken at 200 rpm but the airflow rates were different, namely, 1 and 2 L/min, respectively. Figure 1e and f shows that nisin production was strongly affected by the high airflow rate. In these cultivations, peak values of maximum cell growth and nisin activity were reached at around 16 h, following which the nisin levels dropped sharply, disappearing completely by around 48 h. The maximum cell concentration (X_{max}) reached was 10^7–10^8 CFU/mL until the end of process. The same profile was observed under other culture conditions of this study. Despite the different airflow rate conditions [200 rpm, 1 L/min (k_La=9.72 h^{-1}) and 200 rpm, 2 L/min (k_La=4.32 h^{-1})], the maximum nisin concentration (P_{Nisin}) of 17.70 mg/L (707.95 AU/mL)

reached was equal in both assays. Similar results in terms of maximum nisin productivity (15.77 mg/L, 630.96 AU/mL) were attained with the same agitation (assay 4), but at an aeration rate of 0.5 L/min (k_La=5.96 h^{-1}).

k_La values

k_La values increased with increasing airflow rate and agitation. The maximum value of the concentration gradient reached approximately 1 L/min. Above this value, namely, at an airflow rate of 2 L/min (k_La=4.33 h^{-1}), there was excess gas inside the bioreactor. It is likely that the high airflow rate had a negative effect on oxygen mass transfer. However, the condition without aeration (nearest to microaerophily) suggests higher that the nisin titers are higher and more stable than those ones under the high airflow rate condition.

These considerations suggest that the cells adapted to nisin biosynthesis; however, based on our results, we do not recommend the addition of air during the whole duration of the process. In our study, airflow in the medium appeared to affect nisin production more than biomass production.

pH effect

The decrease in nisin titers may have been caused by cell adsorption or proteolytic degradation. Although nisin-specific protease (nisinase) has been reported in several bacterial strains, including *Streptococcus thermophilus* and *Bacillus cereus*, a conclusive study indicating the presence of nisinase in *L. lactis* has not yet been published. Conversely, adsorption of nisin onto producer cells is a well-established concept and has been reported to be dependent on the pH of the culture broth (Pongtharangku and Demirci 2007). Yang et al. (1992) reported that bacteriocins, in general, have a high adsorption onto cells at pH 6.0 and maximum release from cells at pH 2.0.

Cells convert sugars into lactic acid, thereby acidifying the medium and enhancing the release of nisin from the cells into the medium. In our study, the pH was usually below 5.0 at 12 h into the cultivation process, reaching values from 4.7 upwards at 52 h. Several studies have reported that the optimum pH for bacteriocin production is usually 5.5–6.0 (Cabo et al. 2001; Parente et al. 1994). However, the optimum pH of some bacteriocins has been reported to be lower than 5.0 (Yang and Ray 1994). The optimum pH for bacteriocin production has also been shown to be affected by culture media and species (Cheigh et al. 2002). The influence of milk components on nisin activity was studied by Penna et al. (2005), who found that normal bovine milk at a standard concentration contains about 3.5% protein, which can be fractionated into two main groups. During milk acidification up to pH 4.6 at 20° C, about 80% of the total protein, called casein, precipitates out of solution. Proteins that remains soluble under these conditions are referred to as whey proteins or serum proteins (Robinson 2002). In our study, this phenomenon was observed at the end of the cultivation period or in samples with a pH of <5 (data not shown).

Lactic acid, sugar and proteins

Lactic acid bacteria have complex nutrient requirements because of their limited ability to synthesize B-vitamins and amino acids (Hofvendahl and Hahn-Hägerdal 2000). The amounts of free amino acids required for growth are small, and it is not only assimilated but occasionally decomposed (Carr et al. 1975). Some researchers (Cleveland et al. 2002; Scott and Taylor 1981) have speculated that milk proteins in commercial preparations (Nisaplin and pure nisin) bind nisin, limiting antimicrobial activity. In our study, controls of milk samples revealed no bacteriocin activity (data not shown).

We did not observe any variations in sugar and protein residual consumption by the cells (results not shown). The amount of lactic acid produced using milk sugar (lactose) reached maximum values of approximately 2 mg/mL or 0.2% (Fig. 1a–f), indicating that the lactic acid production was low. Conversely, these results also demonstrate that the dilution factor in skimmed milk was able to reduce the level of surplus nutrients in the medium, promoting cell adaptation and nisin production. This latter finding is extremely important because a substantial proportion of the nutrients seem to remain unconsumed, especially the proteins and peptides, involving superfluous cost. In addition, this could promote difficulties in bacteriocin purification (downstream process).

Conclusions

The results of our study demonstrate that UHT diluted skimmed milk supported both the growth of *L. lactis* cells and the production of bacteriocin by *L. lactis* in batch process. Despite possible variations in the composition of bovine milk due to seasonal and geographical factors, this cultivation medium would appear to have a great potential as a replacement of standard laboratory media, such as MRS or M17 broth, which are expensive and substantially increase the cost of bench- and large-scale nisin production. Low oxygen transfer during the cultivations (microaerophilic condition) and an agitation rate of 200 rpm had a positive effect on nisin release by the microorganism. The dilution factor in skimmed milk was able to diminish the nutrient surplus in the medium, promoting cell adaptation and nisin production. All of these findings are extremely

important in terms of reducing costs and facilitating the downstream process.

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References

Aiba S, Humphrey AE, Millis NF (1973) Biochemical engineering, 2nd edn. University of Tokyo Press, Tokyo

Arauz LJ, Jozala AF, Pinheiro GS, Mazzola PG, Pessoa A Jr, Penna TCV (2008) Nisin expression production from *Lactococcus lactis* in milk whey medium. J Chem Technol Biotechnol 83(3):325–328

Arauz LJ, Jozala AF, Mazzola PG, Penna TCV (2009) Nisin biotechnological production and application: a review. Trends Food Sci Technol 20:146–154

Berlec A, Tompa G, Slapar N, Fonovic UP, Rogelj L, Strukelj B (2008) Optimization of fermentation conditions for the expression of sweet-tasting protein brazzein in *Lactococcus lactis*. Lett Appl Microbiol 46(2):227–231

Bernárdez PF, Amado IR, Castro LP, Guerra NP (2008) Production of a potentially probiotic culture of *Lactobacillus casei* subsp. *casei* CETC 4043 in whey. Int Dairy J 18:1057–1065

Bhatti M, Veeramachaneni A, Shelef LA (2004) Factors affecting the antilisterial effects of nisin in milk. Int J Food Microbiol 97:215–219

Cabo ML, Murado MA, González MP, Pastoriza L (2001) Effects of aeration and pH gradient on nisin production. A mathematical model. Enzyme Microb Technol 29:264–273

Cao LT, Wu JQ, Xie F, Hu SH, Mo Y (2007) Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. J Dairy Sci 90:3980–3985

Carr JG, Cutting CV, Whiting GC (1975) Lactic acid bacteria in beverages and food. Academic, London

Cheigh C-I, Choi H-J, Park H, Kim S-B, Kook M-C, Kim T-S, Hwang J-K, Pyun Y-R (2002) Influence of growth conditions on the production of a nisin-like bacteriocin by *Lactococcus lactis* subsp. *lactis* A164 isolated from kimchi. J Biotechnol 95:225–235

Cleveland J, Chikindas M, Montville TJ (2002) Multimethod assessment of commercial nisin preparations. J Ind Microbiol Biotechnol 29:228–232

Colas J-C, Shi W, Rao VSNM, Omri A, Mozafari MR, Singh H (2007) Microscopial investigation of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting. Micron 38:841–847

De Vuyst L, Vandamme EJ (1992) Influence of the carbon source on nisin production in *Lactococcus lactis* subsp. *lactis* batch fermentations. J Gen Microbiol 138:571–578

Duwat P, Ehrlich SD, Gruss A (1995) The *recA* gene of *Lactococcus lactis*: characterization and involvement in oxidative and thermal stress. Mol Microbiol 17:1121–1131

Duwat P, Sourice S, Cesselin B, Lamberet G, Vido K, Gaudu P, Le Loir Y, Violet F, Loubie're P, Gruss A (2001) Respiration capacity of the fermenting bacterium *Lactococcus lactis* and its positive effects on growth and survival. J Bacteriol 183 (15):4509–4516

Fernández L, Delgado S, Herrero H, Maldonado A, Rodríguez JM (2008) The bacteriocin nisin, an effective agent for the treatment of staphylococcal mastitis during lactation. J Hum Lact 24 (3):311–316

Flôres SH, Alegre RM (2001) Nisin production from *Lactococcus lactis* ATCC 7962 using supplemented whey permeate. Biotechnol Appl Biochem 34:103–107

Furuta Y, Maruoka N, Nakamura A, Omori T, Sonomoto K (2008) Utilization of fermented barley extract obtained from a by-product of barley *shochu* for nisin production. J Biosci Bioeng 106(4):393–397

Garcia-Ochoa F, Gomez E (2009) Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnol Adv 27:153–176

Guerra NP, Castro LP (2002) Production of bacteriocins from *Lactococcus lactis* subsp. *lactis* CECT 539 and *Pediococcus acidilactici* NRRL B-5627 using mussel-processing wastes. Biotechnol Appl Biochem 36:119–125

Guerra NP, Agrasar AT, Macias CL, Pastrana L (2005) Modelling the fed-batch production of pediocin using mussel processing wastes. Process Biochem 40(3–4):1071–1083

Guerra NP, Agrasar AT, Macías CL, Bernárdez PF, Castro LP (2007) Dynamic mathematical models to describe the growth and nisin production by *Lactococcus lactis* subsp. *lactis* CECT 539 in both batch and re-alkalized fed-batch cultures. J Food Eng 82:103–113

Guiga W, Galland S, Peyrol E, Degraeve P, Carnet-Pantiez A, Sebti I (2009) Antimicrobial plastic film: physico-chemical characterization and nisin desorption modeling. Innovative Food Sci Emerg Technol 10(2):203–207

Gupta SM, Aranha CC, Reddy KVR (2008) Evaluation of developmental toxicity of microbicide nisin in rats. Food Chem Toxicol 46(2):598–603

Hofvendahl K, Hahn-Hägerdal B (2000) Factors affecting the fermentative lactic acid production from renewable resources. Enzyme Microb Technol 26:87–107

Jensen NBS, Melchiorsen CR, Jokumsen KV, Villadsen J (2001) Metabolic behavior of *Lactococcus lactis* MG1363 in microaerobic continuous cultivation at a low dilution rate. Appl Environ Microbiol 67(6):2677–2682

Jiang R, Bommarius AS (2004) Hydrogen peroxide-producing NADH oxidase (nox-1) from *Lactococcus lactis*. Tetrahedron Asymmetr 15(18):2939–2944

Jozala AF, Andrade MS, Arauz LJ, Pessoa A Jr, Penna TCV (2007) Nisin production utilizing skimmed milk aiming to reduce process cost. Appl Biochem Biotechnol 136–140:515–528

Liu C, Liu Y, Liao W, Wen Z, Chen S (2004) Simultaneous production of nisin and lactic acid from cheese whey: optimization of fermentation conditions through statistically based experimental designs. Appl Biochem Biotechnol 113–116:627–638

Liu C, Hu B, Liu Y, Chen S (2006) Stimulation of nisin production from whey by a mixed culture of *Lactococcus lactis* and *Saccharomyces cerevisiae*. Appl Biochem Biotechnol 129–132:751–761

López RL, García MT, Abriouel H, Omar NB, Grande MJ, Martínez-Cañamero M, Gálvez A (2007) Semi-preparative scale purification of enterococcal bacteriocin enterocin EJ97, and evaluation of substrates for its production. J Ind Microbiol Biotechnol 34:779–785

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Lv W, Zhang X, Cong W (2005) Modelling the production of nisin by *Lactococcus lactis* in fed-batch culture. Appl Microbiol Biotechnol 68:322–326

Margolles A, Moreno JA, Ruiz L, Marelli B, Magni C, Reyes-Gavilán CG, Ruas-Madiedo P (2009) Production of human growth hormone by *Lactococcus lactis*. J Biosci Bioeng 109(4):322–324

Millette M, Smoragiewicz W, Lacroix M (2004) Antimicrobial potential of immobilized *Lactococcus lactis* subsp. *lactis* ATCC 11454 against selected bacteria. J Food Prot 67(6):1184–1189

Nouaille S, Ribeiro LA, Miyoshi A, Pontes D, De Loir YL, Oliveira SC, Langella P, Azevedo V (2003) Heterologous protein production and delivery systems for *Lactococcus lactis*. Genet Mol Res 2(1):102–111

Ostlie HM, Treimo J, Narvhus JA (2005) Effect of temperature on growth and metabolism of probiotic bacteria in milk. Int Dairy J 15:989–997

Panesar PS, Kennedy JF, Gandhi DN, Bunko K (2007) Bioutilisation of whey for lactic acid production. Food Chem 105:1–14

Parente E, Ricciardi A, Addario G (1994) Influence of pH on growth and bacteriocin production by *Lactococcus lactis* subsp. *lactis* 140NWC during batch fermentation. Appl Microbiol Biotechnol 41:338–394

Penna TCV, Jozala AF, Novaes LCL, Pessoa A Jr, Cholewa O (2005) Production of nisin by *Lactococcus lactis* in media with skimmed milk. Appl Biochem Biotechnol 121–124:619–637

Pereira DBC, Silva PHF, Costa LCG Jr, Oliveira LL (2001) Físico-Química do leite e derivados: métodos Analíticos, 2nd edn. Templo Gráfica e Editora, Juiz de Fora

Pirt J (1975) Principles of microbe and cell cultivation. John Wiley & Sons, New York

Pongtharangkul T, Demirci A (2004) Evaluation of agar diffusion bioassay for nisin quantification. Appl Microbiol Biotechnol 65:268–272

Pongtharangku T, Demirci A (2007) Online recovery of nisin during fermentation and its effect on nisin production in biofilm reactor. Appl Microbiol Biotechnol 74(3):555–562

Robinson RK (2002) Dairy Microbiology Handbook: the microbiology of milk and milk products, 3rd edn. John Wiley & Sons, New York

Sánchez-Manzanares JA, Fernández-Villacañas MR, Marín-Iniesta F, Laencina J (1993) Determination of lactose by an enzymatic method. Food Chem 46:425–427

Scott VN, Taylor SL (1981) Effect of nisin on the outgrowth of *Clostridium botulinum* spores. J Food Sci 46:121–126

Trinetta V, Rollini M, Manzoni M (2008) Development of a low cost culture medium for sakacin A production by *L. sakei*. Process Biochem 43:1275–1280

Vásquez JA, Murado MA (2008) Enzymatic hydrolysates from food wastewater as a source of peptones for lactic acid bacteria productions. Enzyme Microb Technol 43:66–72

Vásquez JA, Cabo ML, González MP, Murado MA (2004) The role of amino acids in nisin and pediocin production by two lactic acid bacteria, A factorial study. Enzyme Microb Technol 34:319–325

Vásquez JA, González MP, Murado MA (2006) Preliminary tests on nisin and pediocin production using waste protein sources. Bioresour Technol 97:605–613

Von Staszewski M, Jagus RJ (2008) Natural antimicrobials: effect of Microgard™ and nisin against *Listeria innocua* in liquid cheese whey. Int Dairy J 18(3):255–259

Yang R, Ray B (1994) Factors influencing production of bacteriocins by lactic acid bacteria. Food Microbiol 11:281–291

Yang R, Johnson MC, Ray B (1992) Novel method to extract large amounts of bacteriocins from lactic acid bacteria. Appl Environ Microbiol 58(10):3355–3359