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ORIGINAL ARTICLE

The effect of carbon and nitrogen sources on bovicin HC5 production by *Streptococcus bovis* HC5

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Abstract

Aims: To investigate the effect of media composition and agroindustrial residues on bovicin HC5 production by *Streptococcus bovis* HC5.

Methods and Results: Batch cultures of *S. bovis* HC5 were grown in basal medium containing different carbon and nitrogen sources. The activity of cell-free and cell-associated bovicin HC5 was determined in culture supernatants and acidic extracts obtained from cell pellets, respectively. *Streptococcus bovis* HC5 produced bovicin using a variety of carbon and nitrogen sources. The highest specific activity was obtained in media containing 16 g l^{-1} of glucose, after 16 h of incubation. The peak in cell-free and cell-associated bovicin HC5 activity was detected when *S. bovis* HC5 cultures reached stationary phase. The bovicin HC5 specific activity and bacterial cell mass increased approximately 3-fold when yeast extract and trypticase (0·5 and 1·0 g l^{-1}, respectively) were added together to the basal medium. *Streptococcus bovis* HC5 cultures produced bovicin HC5 in cheese whey and sugar cane juice and maximal volumetric productivity was obtained after 12 h of incubation.

Conclusions: *Streptococcus bovis* HC5 is a versatile lactic acid bacterium that can utilize several carbon and nitrogen sources for bovicin HC5 production. This bacterium could be a useful model to study bacteriocin production in the rumen ecosystem.

Significance and Impact of the Study: The use of agroindustrial residues as carbon sources could have an economical impact on bovicin HC5 production. To our knowledge, this is the first report to show the use of sugar cane juice for bacteriocin production by lactic acid bacteria.

Introduction

Lactic acid bacteria (LAB) can produce several antimicrobial compounds (e.g. hydrogen peroxide, organic acids and bacteriocins) that inhibit spoilage and pathogenic micro-organisms (Deegan *et al.* 2006). Bacteriocins produced by LAB have received great attention because of their use as 'natural' food preservatives and an increased demand for less-processed and microbiologically safe food products (Gálvez *et al.* 2007). Nisin is one of the most studied bacteriocins due to its spectrum of activity and potential for industrial and medical applications (Delves-Broughton *et al.* 1996; Cleveland *et al.* 2001). However, several reports have demonstrated that some nisin-sensitive bacteria can rapidly become nisin-resistant (Crandall and Montville 1998; Mantovani and Russell 2001; Naghmouchi *et al.* 2007). The development of nisin-resistant strains has increased the interest in studying other bacteriocins with similar spectrum of activity that show stability to heat and acidic conditions.

Bovicin HC5 is a bacteriocin produced by *Streptococcus bovis* HC5 that inhibits the growth of several food-borne and spoilage micro-organisms, including *Listeria monocytogenes* (Mantovani and Russell 2003a), *Bacillus cereus* and *B. thuringiensis* (De Carvalho *et al.* 2007a) and certain species of *Clostridium* (Flythe and Russell 2004; De Carvalho *et al.* 2007b). Bovicin HC5 is a small pore-forming peptide with a unique terminal amino acid

sequence that shows similarity to the lantibiotics (Mantovani *et al.* 2002; Mantovani and Russell 2008). It has stability to high temperatures and acidic pH (Mantovani *et al.* 2002; Houlihan *et al.* 2004), and bovicin HC5-resistant cells have not yet been described. Because of its characteristics, bovicin HC5 could also have potential for industrial application (Russell and Mantovani 2002; Diez-Gonzalez 2007).

Considering that *S. bovis* is an aerotolerant anaerobe with very rapid growth rates and simple nutritional requirements (Wolin *et al.* 1959; Russell and Robinson 1984) fermentation processes for bovicin HC5 production could be performed with low cost substrates and in a short period of time. Previous work indicated that growth conditions could affect bovicin HC5 production and continuous culture experiments indicated that antimicrobial activity was inversely related to the glucose consumption rate (Mantovani and Russell 2003b). These earlier results suggested that the optimization of the growth conditions could improve bovicin HC5 production. The following experiments aimed to: (i) determine the effect of carbon and nitrogen sources on bovicin HC5 production by *Streptococcus bovis* HC5 and (ii) examine the potential for using sugar cane juice and cheese whey as alternative substrates for bacteriocin production.

Materials and methods

Micro-organisms and growth conditions

Streptococcus bovis HC5 was isolated from a cow fed grain (Mantovani *et al.* 2001) and cultivated as previously described (Mantovani and Russell 2003a). *Streptococcus bovis* HC5 was grown in basal media containing (per litre): 292 mg K_2HPO_4, 292 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4{\cdot}7H_2O$, 64 mg $CaCl_2{\cdot}2H_2O$, 500 mg cysteine hydrochloride, 1 g Trypticase, 0·5 g yeast extract and 4 g Na_2CO_3. The medium was prepared anaerobically under an O_2-free carbon dioxide flux and the final pH was adjusted to 6·5 with NaOH (1 mol l^{-1}). Growth and maximal optical densities were determined at 600 nm ($OD_{600\ nm}$) in a Spectronic $20D^+$ (Thermoelectron, Madison, WI, USA). The indicator organism, *Alicyclobacillus acidoterrestris* DSMZ 2498, was grown at 40°C in *Alicyclobacillus acidoterrestris* medium (AAM), described by Yamazaki *et al.* (2000).

Activity of bovicin HC5

Free bovicin HC5 and cell-associated bacteriocin were determined in the cell-free supernatant and in the acidic extract obtained from cell pellets, respectively. Stationary *S. bovis* HC5 cells were harvested by centrifugation (9000 ***g***, 4°C, 15 min) and the culture supernatant was used to determine the activity of free bovicin HC5. The cell pellet was washed (9000 ***g***, 4°C, 15 min) in 10 ml of sodium phosphate buffer (5 mmol l^{-1}, pH 6·7) and re-suspended in a volume of acidic NaCl solution (100 mmol l^{-1}, adjusted to pH 2·0 with 1 mol l^{-1} HCl) that corresponded to 5% of the initial culture volume. Cell suspensions were incubated under agitation (approximately 150 rev min^{-1}) for 2 h at room temperature, followed by centrifugation (9000 ***g***, 4°C, 15 min) and determination of bacteriocin activity in the cell-free extract. Preparations containing bovicin HC5 were serially diluted (twofold increments) into NaCl solution (100 mmol l^{-1}, pH 2·0) and tested for antimicrobial activity against *A. acidoterrestris* DSMZ 2498 using the agar well diffusion technique described by Hoover and Harlander (1993). One arbitrary unit (AU, expressed per ml or cell dry mass) was defined as the reciprocal of the highest dilution that showed a zone of inhibition with at least 5 mm in diameter. When the determination of optical density was not possible due to the turbidity of the media containing sugar cane juice or cheese whey, bovicin activity was expressed as volumetric productivity (AU ml^{-1} h^{-1}).

Effect of carbon and nitrogen sources on bovicin HC5 production

Carbon sources preferentially used by *S. bovis* strains were previously described by Russell and Robinson (1984) and used in this study. The carbon sources tested were glucose, lactose, maltose, mannose, sucrose and cellobiose at concentrations of 4 and 8 g l^{-1}. Batch cultures (3% inoculum, v/v) were grown in anaerobic basal media (50 ml) and kept incubated for approximately 16 h at 39°C. Activity of bovicin HC5 in the supernatant and in the cell extract was tested as described above.

The carbon source that allowed highest bovicin HC5 activity was further tested to evaluate the effect of concentration on bacteriocin production, as indicated in the figure legends. The culture was grown for 16 h and the bovicin HC5 activity in the cell-free culture supernatant was determined for each concentration as described above. In each case, the pH and the $OD_{600\ nm}$ were also determined.

Sugar cane juice and cheese whey were also tested as carbon sources for bacteriocin production by *S. bovis* HC5. Batch cultures were carried out in sealed anaerobic serum bottles (50 ml) or in 500 ml fleaker beaker flasks (Corning) that were continuously purged with O_2-free carbon dioxide. Cheese whey was reconstituted in water, heat-sterilized (121°C, 15 min) and added to twofold concentrated basal media (1 : 1 ratio, v/v). The final

lactose concentration was approximately 24 g l^{-1}. Sugar cane juice (16° Brix) was extracted from ground sugar cane, heat-sterilized (121°C, 15 min) and mixed with basal media (1 : 1 ratio, v/v). The final sugar concentration was approximately 100 g l^{-1}. Samples (20 ml) were taken at time intervals (0, 12, 24 and 48 h) and tested for bacteriocin activity as previously described. Volumetric bacteriocin productivity (Qp, expressed as AU ml^{-1} h^{-1}) was determined from the difference in bovicin activity divided by time (h).

To study the effect of different nitrogen sources on bovicin production, yeast extract and Trypticase were omitted from the basal medium. The basal medium, added with glucose at 16 g l^{-1}, was supplemented with each different nitrogen source (1·5 g l^{-1}). The nitrogen sources tested were yeast extract, Trypticase, meat extract, soy peptone, meat peptone, casein peptone and ammonium sulfate. After 24 h of incubation at 39°C, the final optical densities, culture pH and bacteriocin activity in the culture supernatant and in the cell extract were determined as described above.

Other analyses

Bacterial dry weight was determined by growing *S. bovis* in basal media containing glucose at 16 g l^{-1} (18 h at 39°C) and washing the cells twice in phosphate buffer (5 mmol l^{-1}, pH 6·5). The pellet was concentrated four times and aliquots of 5 ml were dried at 105°C to constant weight. The relationship between optical density (600 nm) and cell dry mass was 360 mg cell dry mass per litre per turbidity unit.

Glucose and fermentation acids in cell-free supernatant were analysed by high-pressure liquid chromatography (Bio-Rad HPX-87H organic acid column). The sample size was 20 μl, the eluant was 0·005 mol l^{-1} H_2SO_4, the flow rate was 0·7 ml min^{-1} and the column temperature was 60°C.

Statistical methods

All experimental determinations were performed in triplicate and the mean, standard deviation and coefficients of variation were computed. The coefficients of variation were always less than 10%. When error bars are given in the figures, they refer to the standard deviation.

Results

Influence of carbon sources on bovicin HC5 production

Batch cultures of *S. bovis* HC5 grew rapidly in basal medium containing mono- or disaccharides and cell mass production increased as the sugar concentration was doubled (Table 1). However, the release of bovicin HC5 in the cell-free supernatant could only be detected in cultures grown in media containing glucose, sucrose or lactose. Cell-associated bovicin HC5 could be extracted from *S. bovis* HC5 cells grown in all the carbon sources tested in this study, but glucose was the preferred sugar for bovicin production (Table 1). Among the disaccharides tested, the highest activity of cell-associated bovicin HC5 was detected in cellobiose-grown cultures (Table 1). Based on these results, glucose was chosen for further characterization of its effect on bovicin HC5 production.

When glucose (up to 160 g l^{-1}) was added to the growth medium, an increase in cell mass and a decrease in culture pH were observed until the sugar concentration was 16 g l^{-1} (results not shown). This increase in cell mass coincided with a greater production of cell-free and

Table 1 Influence of carbon sources on cell growth and bovicin HC5 production by *S. bovis* HC5

Carbon source	Concentration (g l^{-1})	Final pH	Cell dry mass (mg ml^{-1})	Bovicin HC5 specific activity (AU ml^{-1} mg^{-1} dry cell $mass^{-1}$)	
				Cell-free	Cell-associated
Glucose	4	6·18	0·84	160	3048
	8	5·39	0·94	1020	5446
Sucrose	4	6·09	0·72	–	444
	8	4·52	1·08	592	1185
Mannose	4	5·92	0·45	–	1422
	8	4·75	1·30	–	1970
Maltose	4	6·10	0·39	–	410
	8	5·48	1·59	–	805
Cellobiose	4	5·98	0·59	–	1084
	8	5·67	0·54	–	2370
Lactose	4	5·93	0·52	–	1230
	8	5·59	1·04	518	2461

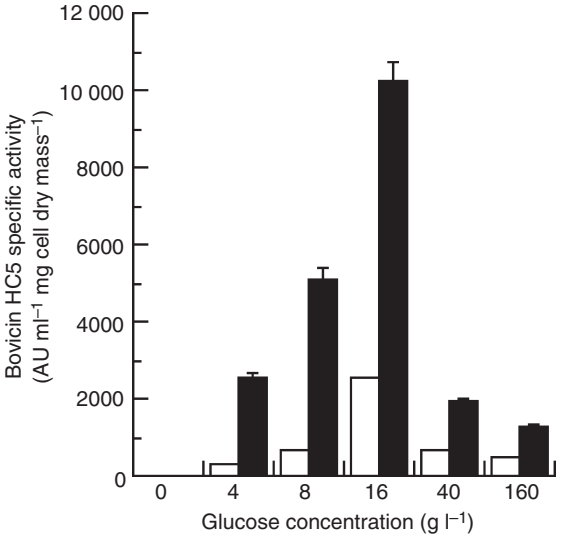

Figure 1 The effect of glucose on bovicin HC5 specific activity. *Streptococcus bovis* HC5 was inoculated into basal medium added with increasing amounts of glucose (0–160 g l^{-1}) and incubated at 39°C for 16 h. Bovicin HC5 activity was determined in cell-free culture supernatants (open bars) and in extracts from *S. bovis* cells treated with acidic NaCl (closed bars).

cell-associated bovicin HC5 (Fig. 1). When glucose concentration was 16 g l^{-1}, *S. bovis* HC5 grew with a specific growth rate of 0·85 h^{-1} and glucose was never completely consumed (Fig. 2a). Bovicin HC5 production reached its peak after *S. bovis* HC5 cultures had reached stationary phase (Fig. 2b). Cell-free bovicin HC5 specific activity was approximately 2500 AU ml^{-1} mg per cell dry mass while the cell-associated activity was nearly four times greater after 24 h of incubation. Cultures grown at higher sugar concentrations showed decreased optical densities (results not shown) and the production of bovicin HC5 was drastically reduced (Fig. 1).

Influence of nitrogen sources on bovicin HC5 production

When *S. bovis* HC5 was cultivated in basal medium that lacked yeast extract and Trypticase and was added with different nitrogen sources (at 1·5 g l^{-1}), a range of cell mass and bovicin HC5 specific activities were obtained (Table 2). The amount of bovicin HC5 detected in the cell extracts was always higher than in the culture supernatants, except when casein peptone was used as nitrogen source (Table 2). The specific activity of cell-free bovicin varied 10-fold among nitrogen sources, while the cell-associated activity varied approximately 24-fold. *Streptococcus bovis* HC5 did not grow well and produced little bacteriocin when the basal medium had ammonium sulfate as the single nitrogen source (Table 2).

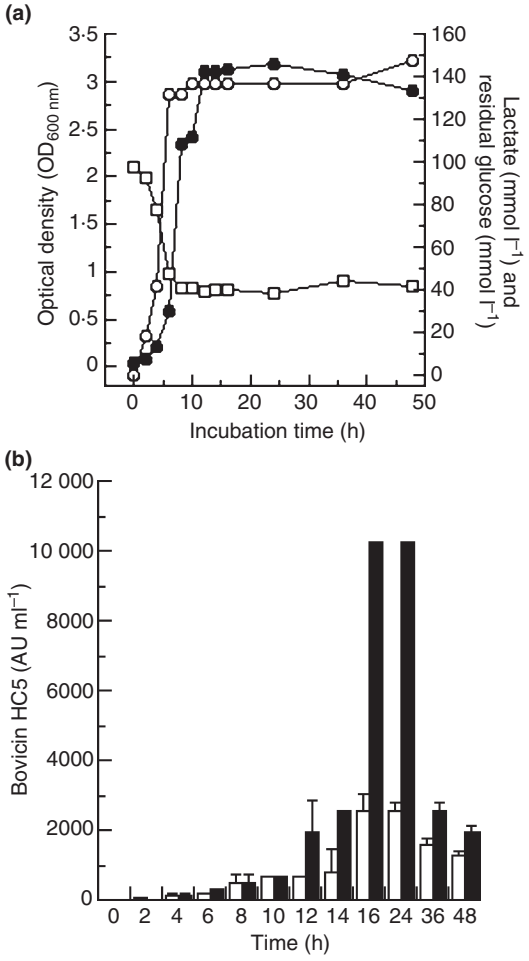

Figure 2 Growth and bovicin production by *S. bovis* HC5 cultivated in basal media containing glucose (16 g l^{-1}) as sole carbon source. Part (a) shows the growth of *S. bovis* (open circles) in basal media, lactate accumulation (closed circles) and residual glucose (open squares). Part (b) shows the activity of cell-free (open bars) and cell-associated (closed bars) bovicin HC5 determined at different time intervals.

When media was added with extra Trypticase and yeast extract (up to 10 g l^{-1}) a positive effect on bacterial cell mass production was observed (results not shown). However, production of bovicin HC5 and cell mass accumulation was not always associated. The production of bovicin by *S. bovis* HC5 was stimulated by yeast extract and Trypticase, but the specific activity of bovicin HC5 decreased if the concentration of yeast extract and Trypticase was greater than 5 and 7 g l^{-1}, respectively (Figs 3 and 4). Interestingly, when optimal concentrations (7 g l^{-1} Trypticase and 1 g l^{-1} yeast extract) of both substrates were added to basal media, an increase in cell mass was observed (1·40 mg ml^{-1} cell dry mass), but the amount of bacteriocin produced was less than observed in basal media alone (data not shown).

Table 2 Influence of nitrogen sources on cell growth and bovicin HC5 production by *S. bovis* HC5

Nitrogen source	Concentration (g l^{-1})	Final pH	Cell dry mass (mg ml^{-1})	Bovicin HC5 specific activity (AU ml^{-1} mg^{-1} - dry cell $mass^{-1}$)	
				Cell-free	Cell-associated
Yeast extract	1·5	4·04	0·88	827	2909
Trypticase	1·5	4·55	0·95	674	1347
Soy peptone	1·5	4·37	0·87	368	552
Meat peptone	1·5	4·66	1·04	769	1230
Casein peptone	1·5	4·33	1·03	466	388
Ammonium sulfate	1·5	5·11	0·36	222	–
Trypticase plus yeast extract	1·0 + 0·5	4·09	1·10	2327	9310

–, Inhibitory activity was not detected.

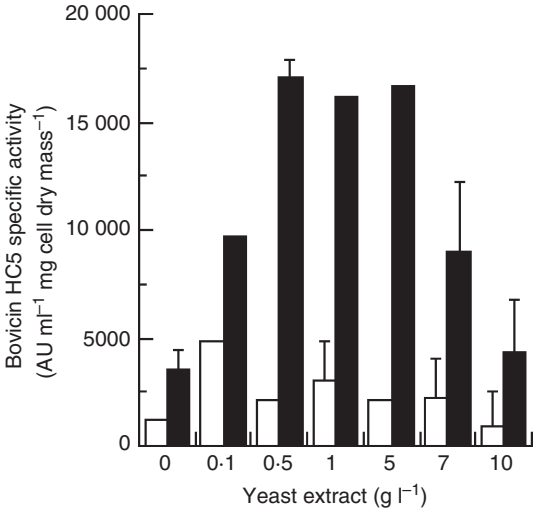

Figure 3 The effect of yeast extract on bovicin HC5 specific activity. Basal medium containing 1 g l^{-1} trypticase was added with increasing yeast extract concentrations (0–10 g l^{-1}) and bovicin HC5 activity in the culture supernatant (open bars) and in the cell extract (closed bars) were determined by the agar well diffusion assay using *A. acidoterrestris* DSMZ 2498 as the indicator organism. *Streptococcus bovis* HC5 cultures were incubated anaerobically for 24 h at 39°C.

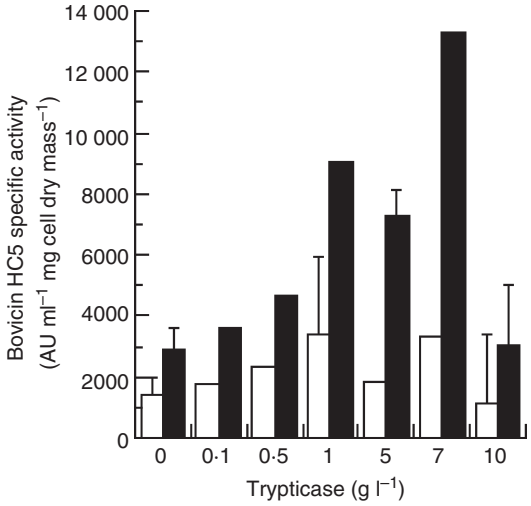

Figure 4 The effect of trypticase on bovicin HC5 specific activity. Basal medium containing 0·5 g l^{-1} of yeast extract and added with increasing trypticase concentrations (0–10 g l^{-1}) and bovicin HC5 activity in the culture supernatant (open bars) and in the cell extract (closed bars) were determined by the agar well diffusion assay using *A. acidoterrestris* DSMZ 2498 as indicator micro-organism. *Streptococcus bovis* HC5 cultures were incubated anaerobically for 24 h at 39°C.

Use of sugar cane juice and cheese whey for bovicin production

Since *S. bovis* HC5 could use sucrose and lactose for bovicin HC5 production, we tested sugar cane juice and cheese whey as potential abundant, low-cost substrates for bacteriocin production. Batch cultures grown in sealed anaerobic bottles had more cell-free bacteriocin than cultures that were continuously flushed with CO_2 for both substrates (Table 3). However, the amount of cell-associated bovicin HC5 was always greater than cell-free, regardless of the condition of incubation. In most cases, maximal volumetric productivity was obtained after 12 h of incubation, and sugar cane juice was the substrate that yielded the highest bovicin HC5 activity (Table 3).

Discussion

LAB have been exploited as a major source of a variety of antimicrobial peptides with potential commercial applications (Cleveland *et al.* 2001; Gálvez *et al.* 2007). LAB are fastidious organisms that often require several organic factors (e.g. amino acids, vitamins) for growth. In order to circumvent their nutritional limitations and stimulate

Table 3 Volumetric productivity (Qp) of bovicin HC5 in media containing sugar cane juice and cheese whey

Incubation time (h)	Substrate							
	Sugar Cane Juice (AU ml^{-1} h^{-1})				Cheese Whey (AU ml^{-1} h^{-1})			
	Under CO_2 flux		Sealed bottles		Under CO_2 flux		Sealed bottles	
	CF	CA	CF	CA	CF	CA	CF	CA
12	53	2560	736	2560	53	2133	426	853
24	53	1706	67	1706	160	853	106	640
48	26	10	53	426	53	33	53	320

CF, cell-free bovicin HC5; CA, cell-associated bovicin HC5.

the production of bacteriocins and other metabolites with applied interest, LAB are frequently cultivated in complex media (Møretrø *et al.* 2000; Kim *et al.* 2006) However, the recovery and purification of bacteriocins can be complicated and costly if the peptide of interest is secreted in media containing contaminating proteins and peptides (Carolissen-Mackay *et al.* 1997; Papagianni *et al.* 2007).

Nonetheless, many *S. bovis* strains can use ammonium salts as their sole nitrogen source (Wolin *et al.* 1959) and simple media can often support *S. bovis* growth and the production of useful metabolites. Bergey's Manual of Determinative Bacteriology defines *Streptococcus bovis* as 'the least nutritionally fastidious species' among streptococci, without a 'requirement for any specific amino acid' (Holt *et al.* 1994) and *S. bovis* HC5, a rapidly growing, gram-positive ruminal bacterium, produces a bacteriocin (bovicin HC5) with broad spectrum of activity (Mantovani *et al.* 2002). Although *S. bovis* HC5 secretes bovicin to the supernatant when the culture pH is low, much of its antimicrobial activity remains cell-associated (Houlihan and Russell 2006a; Xavier *et al.* 2008). Because bovicin HC5 can be released from the producer cells with acidic NaCl, purification can be attained in a single chromatographic step using C-18 reversed-phase columns (Paiva 2007). Therefore, improving bacteriocin production is of great interest for peptide purification, characterization and commercial application.

It has been demonstrated that factors affecting the growth of the producer strain, such as media composition, can improve bacteriocin production. Our results indicated that bovicin production by *S. bovis* HC5 was affected by carbon and nitrogen sources. The highest specific activity of bovicin HC5 was obtained when *S. bovis* was grown in media containing glucose. Several lactic acid bacteria seems to use glucose preferentially for bacteriocin production, and previous studies indicated that *Lactococcus lactis* and *Streptococcus pyogenes* preferred glucose for nisin Z (Matsusaki *et al.* 1996) and streptococcin SA-FF22 production (Jack and Tagg 1992), respectively. In *Lactococcus lactis*, glucose supports higher specific growth rates, faster substrate consumption and greater product formation, compared to other carbon sources (Even *et al.* 2001). Recently, Papagianni *et al.* (2007) indicated a direct relationship between nisin production and the rate of glucose consumption by *L. lactis*.

Early work by Russell and Baldwin (1978) demonstrated that ruminal *S. bovis* uses glucose and sucrose preferentially to maltose and cellobiose and the utilization of these sugars was later shown to be regulated by specific phosphotransferase transport systems (PTS) (Martin and Russell 1987). However, Russell (1990) verified that glucose PTS could not account for the glucose consumption rates of rapidly growing cultures and a low-affinity, facilitated diffusion mechanism was responsible for glucose transport at high substrate concentrations.

These latter results suggest that *S. bovis* can assimilate glucose over a wide range of conditions, which could favour the production of bovicin HC5. However, our results indicated that glucose concentrations greater than 16 g l^{-1} resulted in a decrease in bacterial mass and bovicin activity (Fig. 1). Papagianni *et al.* (2007) observed that batch cultures of *L. lactis* produced less nisin if glucose concentration was above 35 g l^{-1} and this inhibition appeared to be due to a decrease in the rate of glucose uptake. Pattnaik *et al.* (2005) also reported a decrease in bacteriocin production at high glucose concentrations and hypothesized that this inhibition was caused by catabolite repression.

When *S. bovis* HC5 was grown in continuous cultures, the production of bovicin HC5 decreased at high glucose consumption rates and when the culture pH was below 5·4 (Mantovani and Russell 2003b). In our batch cultures, *S. bovis* HC5 produced lactate even if glucose concentration was as high as 200 g l^{-1}. However, specific growth rate decreased rapidly if glucose concentration was above 40 g l^{-1} and the final culture pH was approximately 4·4 (data not shown).

The peak in cell-free and cell-associated bovicin HC5 activity was detected after glucose consumption and lactate production had stopped and *S. bovis* HC5 cultures

reached stationary phase (Fig. 2). However, bovicin HC5 activity decreased after 24 h of incubation (Fig. 2b), even though the culture pH was 4·0. Because Houlihan and Russell (2006b) demonstrated that peptidase activity of *S. bovis* HC5 cultures decreased dramatically at acidic pH values and bovicin HC5 in culture was prevented from being degraded, it appears that the increase in bovicin HC5 activity was due to a greater bacteriocin recovery from *S. bovis* cells. It has been demonstrated that some bovine *S. bovis* strains show competence development and natural genetic transformation (Mercer *et al.* 1999) and changes in cell surface properties and autolytic activity have also been observed. Therefore, it is conceivable that bacteriocin secretion and the recovery of the cell-associated peptide could vary during growth. Further experiments using antibodies raised against bovicin HC5 will address the binding and release of bovicin from *S. bovis* HC5 cells at different growth phases.

Among the single nitrogen sources tested in this study, the highest bovicin HC5 specific activity and bacterial cell mass were observed when yeast extract and trypticase (0·5 and 1·0 g l^{-1}, respectively) were added together to the basal medium of *S. bovis* HC5. Yeast extract is a common source of amino acids and B-complex vitamins in microbiological media and often stimulates bacterial growth and bacteriocin production (De Vuyst 1995; Aasen *et al.* 2000). Trypticase and other sources of amino acids seem to balance catabolic and anabolic rates and increase the specific growth rate and growth yield of ruminal *S. bovis* strains (Russell 1993; Atasoglu *et al.* 1998).

Previous work by Kim *et al.* (2006) indicated that yeast extract and tryptone were the best nitrogen sources for *Micrococcus* sp. GO5 growth and micrococcin GO5 production, respectively. Furthermore, when these nitrogen sources were used together, bacteriocin production increased. *Streptococcus bovis* HC5 produced at least three times more bovicin HC5 when the culture medium had yeast extract and trypticase combined compared to the use of each source alone (Table 2). Because cell mass production and bacteriocin activity are not readily related, the effect of nitrogen sources on bovicin HC5 production appears to involve multiple physiological responses.

Because some agroindustrial wastes can be used as alternative substrates to sustain bacterial growth and stimulate bacteriocin production, we tested the potential of sugar cane juice and cheese whey to improve bovicin HC5 activity. Cheese whey is a highly polluting, lactose-rich byproduct from cheese industries that has been used for bacteriocin production (Carolissen-Mackay *et al.* 1997; Alvarez *et al.* 2006). Sugar cane juice has been commonly used for fuel ethanol fermentation, production of sugar cane spirits and metabolites of industrial interest. However, bacteriocin production on sugar cane juice based media had not been demonstrated.

Our results indicated that *S. bovis* HC5 cultures produced more bovicin HC5 in cheese whey and sugar cane juice than in basal medium, and this observation suggest that bacteriocin production costs could be lowered using these abundant agroindustrial substrates. Because our batch cultures always had more cell-associated than cell-free bacteriocin, the recovery of purified bovicin HC5 could be improved even if the cultures were grown in these complex, low-cost media. The observation that *S. bovis* had more bovicin HC5 activity when sugar cane juice was used as substrate is probably due to the fact that media containing sugar cane juice provided approximately four times more carbon and energy sources than cheese whey. To our knowledge, this is the first report of the use of sugar cane juice for bacteriocin production.

As previous studies demonstrated that bovicin HC5 has great potential for agricultural and industrial applications, *S. bovis* HC5 might be a useful model to study bacteriocin production. Considering that *S. bovis* HC5 is a versatile lactic acid bacterium that can utilize several carbon and nitrogen sources for bovicin HC5 production, high bacteriocin yields can be attained using alternative substrates. Further studies are being conducted to investigate the effect of physical–chemical factors (e.g. pH, temperature and reduction potential) on bovicin HC5 production and to evaluate its toxicity against animal cell lines.

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References

Aasen, I.M., Møretrø, T., Katla, T., Axelsson, L. and Storro, I. (2000) Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687. *Appl Microbiol Biotechnol* **53**, 159–166.

Alvarez, D.C., Pérez, V.H., Justo, O.R. and Alegre, R.M. (2006) Effect of the extremely low frequency magnetic field on nisin production by *Lactococcus lactis* subsp. *lactis* using cheese whey permeate. *Process Biochem* **41**, 1967–1973.

Atasoglu, C., Valdes, C., Walker, N.D., Newbold, C.J. and Wallace, R.J. (1998) De novo synthesis of amino acids by the ruminal bacteria *Prevotella bryantii* B14, *Selenomonas ruminantium* HD4, and *Streptococcus bovis* ES1. *Appl Environ Microbiol* **64**, 2836–2843.

Carolissen-Mackay, V., Arendse, G. and Hastings, J.W. (1997) Purification of bacteriocin of lactic acid bacteria: problems and pointers. *Int J Food Microbiol* **34**, 1–16.

Cleveland, J., Montville, T.J., Nes, I.F. and Chikinda, M.L. (2001) Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol* **71**, 1–20.

Crandall, A.D. and Montville, T.J. (1998) Nisin resistance in *Listeria monocytogenes* ATCC 700302 is a complex phenotype. *Appl Environ Microbiol* **64**, 231–237.

De Carvalho, A.A.T., Costa, E.D., Mantovani, H.C. and Vanetti, M.C.D. (2007a) Effect of bovicin HC5 on growth and spore germination of *Bacillus cereus* and *Bacillus thuringiensis* isolated from spoiled mango pulp. *J Appl Microbiol* **102**, 1000–1009a.

De Carvalho, A.A.T., Mantovani, H.C. and Vanetti, M.C.D. (2007b) Bactericidal effect of bovicin HC5 and nisin against *Clostridium tyrobutyricum* isolated from spoiled mango pulp. *Lett Appl Microbiol* **45**, 68–74b.

De Vuyst, L. (1995) Nutritional factors affecting nisin production by *Lactococcus lactis* subsp. *lactis* NIZO 22186 in a synthetic medium. *J Appl Bacteriol* **78**, 28–33.

Deegan, L.H., Cotter, P.D., Hill, C. and Ross, P. (2006) Bacteriocins: biological tools for bio-preservation and shelf-life extension. *Int Dairy J* **16**, 1058–1071.

Delves-Broughton, J., Blackburn, P., Evans, R.J. and Hugenholtz, J. (1996) Applications of the bacteriocin, nisin. *Antonie Van Leeuwenhoek* **69**, 193–202.

Diez-Gonzalez, F. (2007) Applications of bacteriocins in livestock. *Curr Issues Intest Microbiol* **8**, 15–23.

Even, S., Lindley, N.D. and Cocaign-Bousquet, M. (2001) Molecular physiology of sugar catabolism in *Lactococcus lactis* IL1403. *J Bacteriol* **183**, 3817–3824.

Flythe, M.D. and Russell, J.B. (2004) The effect of pH and a bacteriocin (bovicin HC5) on *Clostridium sporogenes* MD1, a bacterium that has the ability to degrade amino acids in ensiled plant materials. *FEMS Microbiol Ecol* **47**, 215–222.

Gálvez, A., Abriouel, H., López, R.L. and Ben Omar, N. (2007) Bacteriocin-based strategies for food biopreservation. *Int J Food Microbiol* **120**, 51–70.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994) *Bergey's Manual of Determinative Bacteriology*, 9th edn. pp. 789. Baltimore, USA: The Williams & Wilkins Co.

Hoover, D.G. and Harlander, S.K. (1993) Screening methods for detecting bacteriocin activity. In *Bacteriocins of Lactic Acid Bacteria* ed. Hoover, D.G. and Steenson, L.R. pp. 23–39. San Diego, CA: Academic Press.

Houlihan, A.J. and Russell, J.B. (2006a) The effect of calcium and magnesium on the activity of bovicin HC5 and nisin. *Curr Microbiol* **53**, 365–369.

Houlihan, A.J. and Russell, J.B. (2006b) Factors affecting the activity of bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5: release, stability and binding to target bacteria. *J Appl Microbiol* **100**, 168–174.

Houlihan, A.J., Mantovani, H.C. and Russell, J.B. (2004) Effect of pH on the activity of bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5. *FEMS Microbiol Lett* **231**, 27–32.

Jack, R.W. and Tagg, J.R. (1992) Factors affecting production of the group A streptococcus bacteriocin SA-FF22. *J Med Microbiol* **36**, 132–138.

Kim, M.H., Komg, Y.J., Baek, H. and Hyun, H.H. (2006) Optimization of culture conditions and medium composition for the production of micrococcin GO5 by *Micrococcus* sp. *GO5. J Biotechnol* **121**, 54–61.

Mantovani, H.C. and Russell, J.B. (2001) Nisin resistance of *Streptococcus bovis*. *Appl Environ Microbiol* **67**, 808–813.

Mantovani, H.C. and Russell, J.B. (2003a) Inhibition of *Listeria monocytogenes* by bovicin HC5, a bacteriocin produced by *Streptococcus bovis* HC5. *Int J Food Microbiol* **89**, 77–83.

Mantovani, H.C. and Russell, J.B. (2003b) Factors affecting the antibacterial activity of the ruminal bacterium, *Streptococcus bovis* HC5. *Curr Microbiol* **46**, 18–23.

Mantovani, H.C. and Russell, J.B. (2008) Bovicin HC5, a lantibiotic produced by *Streptococcus bovis* HC5, catalyzes the efflux of intracellular potassium but not ATP. *Antimicrob Agents Chemother* **52**, 2247–2249.

Mantovani, H.C., Kam, D.K., Ha, J.K. and Russell, J.B. (2001) The antibacterial activity and sensitivity of *Streptococcus bovis* strains isolated from rumen of catle. *FEMS Microbiol Ecol* **37**, 223–229.

Mantovani, H.C., Hu, H., Worobo, R.W. and Russell, J.B. (2002) Bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5. *Microbiology* **148**, 3347–3352.

Martin, S.A. and Russell, J.B. (1987) Transport and phosphorylation of disaccharides by ruminal bacterium *Streptococcus bovis*. *Appl Environ Microbiol* **53**, 2388–2393.

Matsusaki, H., Endo, N., Sonomoto, K. and Ishizaki, A (1996) Lantibiotic nisin Z fermentative production by *Lactococcus lactis* 10-1: relationship between production of the lantibiotic and lactate and cell growth. *Appl Microbiol Biotechnol* **45**, 36–40.

Mercer, D.K., Melville, C.M., Scott, K.P. and Flint, H.J. (1999) Natural genetic transformation in the rumen bacterium *Streptococcus bovis* JB1. *FEMS Microbiol Lett* **179**, 485–490.

Møretrø, T., Aasen, I.M., Storrø, I. and Axelsson, L. (2000) Production of sakacin P by *Lactobacillus sakei* in a completely defined medium. *J Appl Microbiol* **88**, 536–545.

Naghmouchi, K., Kheadr, E., Lacroix, C. and Fliss, I. (2007) Class I/Class IIa bacteriocin cross-resistance phenomenon in *Listeria monocytogenes*. *Food Microbiol* **24**, 718–727.

Paiva, A.D. (2007) *Produção de Anticorpos policlonais para a detecção de bovicina HC5 por ensaios imunoenzimáticos*. Pp. 93. Viçosa: Universidade Federal de Viçosa. Dissertação de Mestrado.

Papagianni, M., Avramidis, N. and Filioussis, G. (2007) Investigating the relationship between the specific glucose uptake rate and nisin production in aerobic batch and fed-batch glucostat cultures of *Lactococcus lactis*. *Enzyme Microb Technol* **40**, 1557–1563.

Pattnaik, P., Grover, S. and Batish, V.K. (2005) Effect of environmental factors on production of lichenin, a chromosomally encoded bacteriocin like compound produced by *Bacillus licheniformis* 26L-10/3RA. *Microbiol Res* **160**, 213–218.

Russell, J.B. (1990) Low-affinity, high-capacity system of glucose transport in the ruminal bacterium *Streptococcus bovis*: evidence for a mechanism of facilitated diffusion. *Appl Environ Microbiol* **56**, 3304–3307.

Russell, J.B. (1993) Effect of amino acids on the heat production and growth efficiency of *Streptococcus bovis*: balance of anabolic and catabolic rates. *Appl Environ Microbiol* **59**, 1747–1751.

Russell, J.B. and Baldwin, R.L. (1978) Substrate preferences in rumen bacteria: evidence of catabolic regulatory mechanisms. *Appl Environ Microbiol* **37**, 531–536.

Russell, J.B. and Mantovani, H.C. (2002) The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. *J Mol Microbiol Biotechnol* **4**, 347–355.

Russell, J.B. and Robinson, P.H. (1984) Compositions and characteristics of strains of *Streptococcus bovis*. *J Daity Sci* **67**, 1525–1531.

Wolin, M.J., Manning, G.B. and Nelsin, W.O. (1959) Ammonia salts as a sole source of nitrogen for the growth of *Streptococcus bovis*. *J Biotechnol* **13**, 269–272.

Xavier, B.M., Houlihan, A.J. and Russell, J.B. (2008) The activity and stability of cell-associated activity of bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5. *FEMS Microbiol Lett* **283**, 162–166.

Yamazaki, K., Murakami, M., Kawai, Y., Inoue, N. and Matsuda, T. (2000) Use of nisin for inhibition of *Alicyclobacillus acidoterrestris* in acidic drinks. *Int J Food Microbiol* **17**, 315–320.