International Journal of Dairy Science and Technology Vol. 1 (4), pp. 061-068, December, 2014. Available online at www.advancedscholarsjournals.org © Advanced Scholars Journals

Full Length Research Paper

Fractional purification and characterization of two bacteriocin-like inhibitory substances produced by bifidobacteria

Latifah Delanoe

Department of Animal Science, Faculty of Agricultural Science, Tunis University, Tunis, Tunisia.
E-mail: latifdela@yahoo.co.uk

Accepted 01 November, 2014

Bifidobacterium **spp. RBL 68 and RBL 85 isolated from newborn faeces were found to produce two bacteriocin-like inhibitory substances (BLIS) with inhibitory activities against a wide range of Gram-positive and Gram-negative bacteria. The production of these BLIS began in late exponential phase of growth and reached a maximum activity during and after the stationary phase. An activity level of 33 and 15 AU/ml at the end of the exponential phase that is (12 h) and maximum activity (65 and 35 AU/ml) at the beginning of the stationary phase that is (24 and 36 h) were recorded in MRS broth at 37°C for RBL 68 and RBL 85, respectively. The two BLIS, produced by RBL 68 and RBL 85, were partially purified by a three-step purification protocol resulting in a specific activity of 2.66 x 10^3 and 7.68 x 10^3 AU/mg and purification fold of 122.8 and 95, respectively. Complete inactivation of the two BLIS activities were observed after treatment with proteolytic enzymes, including chymotrypsin, pronase E and proteinase K. These proteinaceous compounds were active against food- borne diseases and food spoilage pathogens such as *Listeria monocytogenes*, which make them potentially useful as antimicrobial agents in foods.**

Key words: *Bifidobacterium* spp., bacteriocin-like inhibitory substance, bacteriocin-like inhibitory substances activity.

INTRODUCTION

Bifidobacterium is one of the most common genus in the human intestinal microbiota. *Bifidobacterium* constitutes up to 25% of the total population in the intestinal tract in adults and 95% in newborns (Yildirim and Johnson, 1998). One positive effect of *Bifidobacterium* in the human microflora is the production of antimicrobial compounds other than organic acids, such as bacteriocins. While very few reports exist on the production of bacteriocins from *Bifidobacterium sp*. (Cheikhyoussef et al., 2009, 2010; Yildirim et al., 1999; Yildirim and Johnson, 1998). Klaenhammer (1993) reported that 99% of all bacteria may make at least one bacteriocin and the only reason we have not isolated more is that few researchers have looked for them (Klaenhammer, 1988). Some studies have attributed the inhibitory effect of bifidobacteria to the production of antimicrobial proteinaceous compounds (Gong et al., 2010; Meghrous et al., 1997). To date, some bacteriocins such as bifidin I (Cheikhyoussef et al., 2010), bifidocin B (Yildirim et al., 1999) and bacteriocin-like inhibitory substances (BLIS) (Cheikhyoussef et al., 2009; Collado et al., 2005; Toure et al., 2003) have been reported to be produced by Bifidobacteria. To our knowledge, Bifidocin B and bifidin I are the only bacteriocins produced by

Table 1. Bacterial reference strains used in this study and their sensitivity to RBL68 and RBL85 BLIS.

Organism	Strain	Sensitivity to (RBL85)	Sensitivity to (RBL68)
L. rhamnosus	R [a]0011	+ +	+
S. thermophilus	R0083	+ + +	+
P. freudenreichii	R0501	+	+
L. delb. bulgaricus	66	+ +	-
Propionibacterium spp.	*p* 5	+ +	+
P. acidilacticii	R1001	+ +	+
P. acidilactici	R47	+ +	+
P. acidipropionici	DH42	+	-
P. freudenreichii	p 63	+	-
B. longum	R0175	+ + +	-
L. casei	R0256	+ +	+
L. lactis subsp.*lactis*	R 0058	+ + +	+ +
L. del. lactis	R0187	+ +	-
L. salivarius	R0078	+	-
B. breve	R0070	+ +	-
B. animalis	ATCC [b]27536	+	+
B. breve	ATCC15700	+	+
L. monocytogenes	LSDCC [c]538-4bx	+ + +	+ +
L. monocytogenes	LSDCC529-3a	+ + + +	+ +
L. moncytogenes	LSDCC530-3b	+ + + +	+ +
B. bifidum	ATCC15696	+ + +	+ +
B. adolescentis	ATCC 15704	+ +	+
P. acidipropionici	EQU2	+ +	-
Propionibacterium	R1042	+ +	+
Propionibacterium	R1042	+ +	+
E. faecium	R0026	+ +	+
S. typhimurium	ATCC14028	+ +	+
P. acidipropionici	RDH42	+ +	+
E. coli	ATCC11775	+ +	+ +

-, no inhibition., + inhibitory activity 5-10 mm, + + inhibitory activity 10-15 mm, + + + inhibitory activity 15-20 mm, + + + + inhibitory activity > 20 mm, [a]R: Rossell: Institute Inc .(Montreal, Quebec, Canada), [b]ATCC: Americain type Culture collection (Rockville, MD, USA) and [c]LSDCC: Laboratory Services Division culture collection.

bifidobacteria that have been purified and characterized and found to inhibit growth of species of *Listeria, Enterococcus, Bacillus, Lactobacillus* and *Pediococcus* (Yildirim et al., 1999; Yildirim and Johnson, 1998; Cheikhyoussef et al., 2010).

Using bacteriocins to improve the microbial quality and safety of food has stimulated intensive research efforts in recent years. Bacteriocins produced by lactic acid bacteria have been evaluated in the preservation of milk, meat, and vegetables due to their capacity to inhibit the growth of pathogenic- spoilage that causes bacteria. In previous work, Toure et al. (2003) have studied the antimicrobial activity from infant bifidobacterial strains RBL 68 and RBL 85. In this work, we report the antimicrobial activity as well as the characterization and partially purification of two BLIS produced by the two bifidobacterial strains RBL 68 and 85 with wide inhibitory spectrum including a group of food borne pathogens.

MATERIALS AND METHODS

Bacteria strains and growth media

Reference bacterial strains used in this study and their origins are listed in Table 1. All strains were maintained in 20% glycerol at –80°C. *Lactococcus* spp. were grown in de Man, Rogosa and Sharpe (MRS) broth obtained from Rosell Institute Inc. (Montréal, PQ, Canada) containing 0.1% (v/v) Tween 80 and incubated aerobically at 30°C. *Salmonella* and *Escherichia coli* were grown in tryptic soy broth (TSB; Difco Laboratories, Sparks, MD) supplemented with 0.6% (w/v) yeast extract and incubated aerobically at 37°C. *Listeria monocytogenes* and *Listeria ivanovii* were grown in TSB with yeast extract and incubated aerobically at 30°C.

Streptococcus thermophilus, Enterococcus, and pediococci were grown in MRS broth at 37°C under aerobic conditions. All lactobacilli, propionibacteria and bifidobacteria were grown in MRS broth supplemented with 0.05% (w/v) L-cysteine-hydrochloride (Sigma Chemical Co., St. Louis, MO, USA) and incubated anaerobically under an atmosphere generated using the

OxoidAnaeroGen™ System (Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C. Before the experiments, strains were sub-cultured at least three times, in their respective media at 24 h intervals. *L. ivanovii* was chosen as the indicator strain for antimicrobial assays.

Production of BLIS from *Bifidobacterium* spp. RBL 68 and RBL 85

Growth and BLIS production by strains RBL 68 and RBL 85 in MRS broth (initial pH 6) (1%, v/v) were followed during 24 h of incubation at 37°C under anaerobic conditions using an inoculation level of 1% (v/v). Viable bacterial counts, BLIS activities and pH were determined at 3 h intervals. For BLIS titer determination, 5 ml of culture supernatants were separated by centrifugation prior to assay by the critical-dilution micromethod described below.

Two-fold serial dilutions of 125 µl of tested sample were added to wells of a flat bottomed microtest™ polystyrene microplate (96-well microtest, Becton Dickinson Labware, FranklinLakes, NJ, USA). Each well contained 125 µl of MRS- c broth at 1% (vol/vol) (Meghrous et al., 1997). Each well was inoculated with 50 µl of 1000-fold diluted overnight culture of each indicator strain (final concentration of approximately 10^6 CFU/ml). Plates were incubated at 37°C for 18 h and the optical density (OD) at 650 nm was then measured using a Thermo-max molecular device spectrophotometer (OPTI-Resources Inc., Québec, PQ, Canada). Crude BLIS activity, expressed as arbitrary units per milliliter (AU/ml), was defined as the highest BLIS dilution showing complete inhibition of the indicator strain (OD_{650} equal to that in uninoculated medium), calculated as $AU/ml=2^n$ x (1000/125), where n is the number of wells showing inhibition of the indicator strain.

Effect of enzymes on *Bifidobacterium* spp. RBL 68 and RBL 85 BLIS

To confirm the proteinaceous nature of the inhibitory substances, the crude BLIS extracts were incubated at 37°C for 18 h in the presence of proteolytic enzymes (catalase, pepsin, -chymotrypsin, trypsin, protease, pronase E and proteinase K, all from Sigma). Enzymes were dissolved in 0.01 M phosphate buffer saline (PBS) (Sigma) at pH 7 at a concentration of 10 mg/ml.

Temperature and pH stability

Temperature stability was determined by measuring the activity of RBL 68 and RBL 85 BLIS after treatment at 100°C for 15 and 30 min and by autoclaving at 121°C for 15 min.

The pH stability was determined by adjusting samples of RBL 68 and RBL 85 BLIS to different pH 2, 4, 6 and 8.

Spectrum of activity

The antibacterial activity of the crude BLIS against several bacterial species was evaluated using the agar diffusion method (Tagg et al., 1976); the pH of the supernatant was adjusted to 6.5 with 5 M of NaOH; strains used as indicators are reported in Table 1.

Partial purification of the two BLIS

Partial purification of the two BLIS was performed using a three-step method adapted from Guyonnet et al. (2000). Overnight MRS cultures of strainsRBL 68 and RBL 85 were centrifuged at 7500 x g for 20 min at 4°C and the supernatants were sterilized by filtration through 0.45 m-sizes and lyophilized pore filters. After freeze-drying of the two supernatants, the extraction of the BLIS was made using methanol and acetone. The extracts (25 ml) were injected into a SP-Sepharose fast flow cation exchange column (Amersham, Pharmacia Biotech, Uppsala, Sweden) at a flow rate of 3 ml/min. The column was washed and equilibrated with 500 ml of sodium phosphate buffer (0%NaCl, pH 6) . The two BLIS RBL 68 and RBL 85 were eluted with 50 ml of 1% (w/v) and 5% sodium chloride in sodium phosphate buffer respectively. The eluted BLIS were loaded onto a Sep-Pack® Cig Cartridge micro-column (Waters, Milford, Massachusetts, USA) previously equilibrated with 5 mM of HCl. The two BLIS were eluted from the Sep-Pack using 30 ml of 50% (v/v) acetonitrile in water. Acetonitrile was removed using a rotary evaporator.

Protein concentration was determined using the DC protein assay (Bio-Rad Laboratories, Mississauge, ON, Canada) and bovine serum albumin (Pierce Chemical Compagny, Rockford, IL, USA) as standard (Mathieu et al., 1993). At each purification step, BLIS activity was assayed by the critical dilution micromethod as described above.

RESULTS

Activity spectrum

The pH of the two BLIS was adjusted to pH 6.5 while inhibitory effects on various bacterial strains were tested. Results are reported in Table 1. The inhibitory spectrums of the two BLIS were quite broad, including Gram - negative and Gram-positive pathogenic bacterial strains.

Preliminary characterization of BLIS

It is worth to note that the inhibitory activity disappeared in the presence of proteinase for RBL 68 BLIS and -chymotrypsin, pronase E and proteinase K for RBL 85 extract. Catalase had no effect on the activity. The two BLIS retained a considerable portion of their activity after high temperature treatments, as determined by the agar diffusion method. The activity of the inhibitory substances was maintained at 100°C after 15 and 30 min and following autoclaving at 121°C for 15 min. The BLIS of the two strains were active in a wide pH range from 2 to 6.

Kinetics of BLIS production

Kinetic of the two BLIS production in MRS broth is shown in Figures 1 and 2. The two strains grew satisfactorily in MRS broth at 37°C, the maximum of viable cell count reached approximately 6.10 and 5.10 log CFU/ml for *Bifidobacterium* spp. RBL 68 and RBL 85, respectively after 18 h.

Production of the two BLIS began during the late stage of exponential growth. Acid production appeared to be growth-associated since most of it was observed towards the end of the exponential growth phase where the pH decreased from 6.5 to 4 while this latter remained relatively stable during and after the stationary phase.

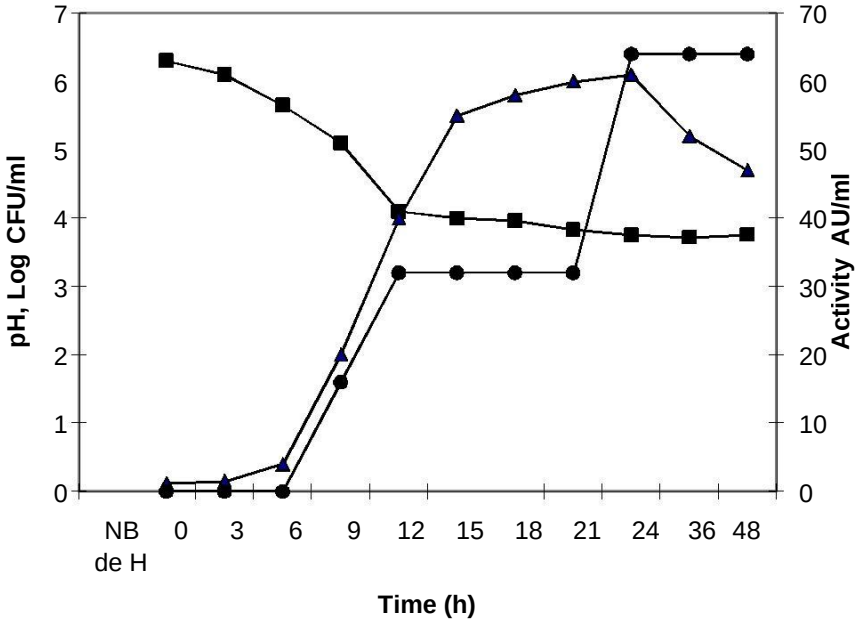

Figure 1. Growth of *Bifidobacterium* spp. RBL 68 (), BLIS activity (- -) and acid production (- -) in De Man, Rogosa and Sharpe (MRS) broth at 37°C.

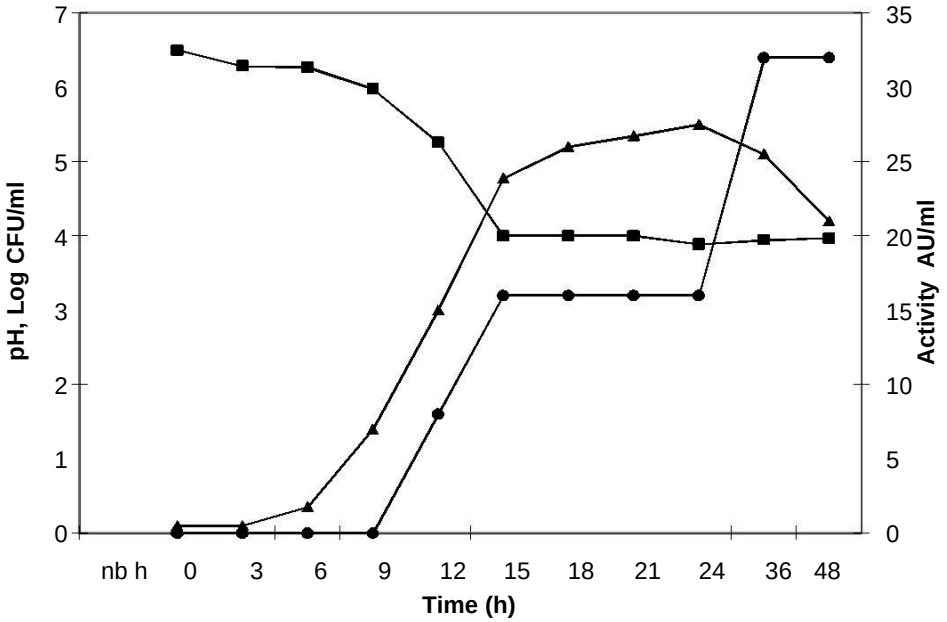

Figure 2. Growth of *Bifidobacterium* spp. RBL 85 ( ), BLIS activity (- -) and acid production (- -) in De Man, Rogosa and Sharpe (MRS) broth at 37°C.

Partial purification of the two BLIS

Bifidobacterium spp. RBL 68 and 85- the strains isolated from faeces of a newborn produced BLIS that were partially purified. Changes in sample's biological activity and overall purity following each purification step are summarized in Tables 2 and 3. Figure 3 shows the activity of BLIS produced by *Bifidobacterium* spp. RBL 85 obtained at different steps of the partial purification procedures. Based on activity measurement, only 38.50 and 11.42% of the RBL 68 and RBL 85 BLIS activities present in the cell-free supernatants were eluted from methanol and acetone extraction respectively (Tables 2 and 3). Moreover, it was found that 57.5 and 45.7% of the

Table 2. Partial purification steps of BLIS produced by *Bifidobacterium* spp. RBL68.

Purification stage	Volume (ml)	Total protein (mg)	Total activity (UA/ml)	Spécific activity (UA/mg)	Purification fold	Yield (%)
Culture supernatant	175	1032	2.24×10^4	21.7	-	100
Methanol-acetone extract	5	126	1.28×10^3	10.15	1.2	38.5
Sp-Sepharose eluate	50	69	1.28×10^4	1.85×10^2	8.5	57.1
Sep-Pack C18 eluate	150	7.2	1.92×10^4	2.6666×10^3	122.8	85.7

Table 3. Partial purification steps of BLIS produced by *Bifidobacterium* spp. RBL85.

Purification stage	Volume (ml)	Total Protein (mg)	Total Activity (UA/ml)	Spécific activity (UA/mg)	Purification fold	Yield (%)
Culture supernatant	175	1102.5	2.24×10^4	21.7	-	100
Methanol-acetone extract	5	120.2	2.56×10^3	21.29	1.05	11.42
Sp-Sepharose eluate	40	17.2	10.24×10^3	5.95×10^2	29.75	45.7
Sep-Pack C18 eluate	120	4	7.68×10^3	1.92×10^3	95	34.3

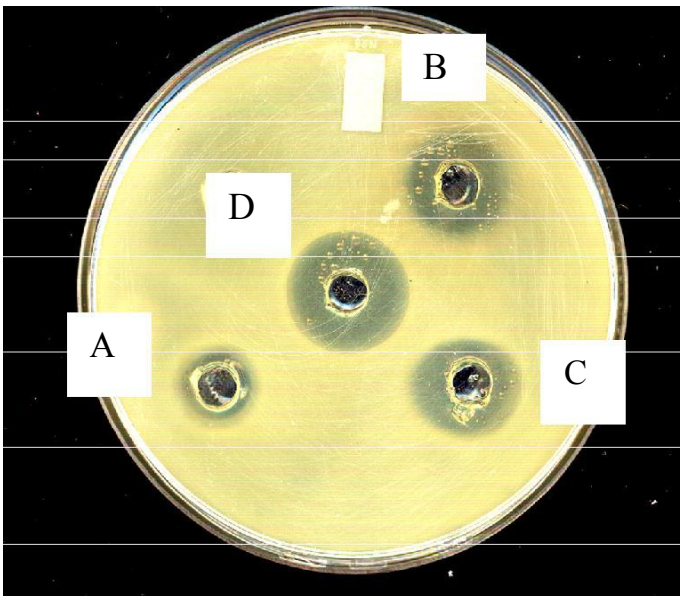

Figure 3. Agar-well diffusion showing the inhibition of *Listeria ivanovi* by BLIS from *Bifidobacterium* spp. RBL 85 culture supernatant (A), extract methanol acetonic (B), 5% sodium chloride eluate from SP- Sepharose (C) and column eluate from Sep-Pack Cl8 column with 50% acetonitrile (D), respectively.

BLIS produced, respectively, by RBL 68 and RBL 85 were eluted from the SP-Sepharose cation-exchange column. The two BLIS eluted from SP-Sepharose column were further purified on a Sep-Pack C18 column. They bound tightly to the column matrix but could be easily eluted with 50% (v/v) acetonitrile. The Sep-Pack C18 separation increased the specific activities of BLIS of *Bifidobacterium* spp. RBL 68 and RBL 85 by 122.8 and 95-fold, respectively. The recovered amounts of RBL 68 and RBL 85 present in the crude supernatants were 85.7 and 34.3, respectively.

DISCUSSION

It was observed that the inhibitory activity disappeared in the presence of proteinase for RBL 68 extract and -chymotrypsin, pronase E and proteinase K for RBL 85

extract. This observation indicates that the inhibitory materials in the two BLIS were perteinaceous. It was noticed that the antagonistic activity of two *Bifidobacterium* spp. RBL 68 and 85 is due to the production of BLIS. The partially purified BLIS are relatively heat-stable. The BLIS have heat stability comparable to that of bifidocin B (Yildirim and Johnson 1998) and BLIS (Cheikhyoussef et al., 2009). The heat stability is a very useful characteristic in the application of bacteriocin or BLIS as a food preservative, because many food processing procedures involve a heating step.

The supernatants of the two strains were active at pH 2 to 6 such as lactocin S and leucocin A-LAU-187 which are active at a pH below 5.5 and between 2 and 3 respectively (De Kwaadsteniet et al., 2005). The heat and pH stability of bacteriocins from bifidobacteria enhance the resistance of the BLIS to food processing technologies, as high acidity is believed to be the most detrimental factor affecting the viability of bifidobacteria in fermented foods (Cheikhyoussef et al., 2009).

The highest number of cells was observed after 18 h of incubation while the number of the cell counts started to decrease after 24 h for the two strains. This finding is in agreement with the bacteriocins production data from LAB (1, 11, 19, 15, 18, 28). Biologically active crude BLIS of RBL 68 and 85 strains were first detected after 9 and 12 h of growth (approximately 18 and 10 AU/ml) respectively. This activity reached a maximum of 65 and 34 AU/ml after 24 and 36 h of growth respectively which corresponded to the mortality phase of the two strains. This result suggested that the production of BLIS was dependent on the cell number under these growth conditions. During extended incubation time at the stationary phase, the activity increased considerably. Like most bacteriocins (Pilet et al., 1995), RBL 68 and 85 BLIS were produced in the late exponential phase of growth. Concentrations of RBL 68 and RBL 85 reached, respectively, a maximum level after 24 and 36 h of incubation. As such, it is suggested that the production or study of the two BLIS should start after 24 h for RBL 68 strain and after 36 hours for RBL 85 strain.

Although, the importance of pH adjustment in MRS broth for growth and BLIS production has been shown in various studies (Mathieu et al., 1993; Schillinger et al., 1993; Holck et al., 1996), growth of *Bifidobacterium* spp. RBL 68 and 85 and BLIS production in MRS broth was not affected by the initial pH. The two BLIS produced *Bifidobacterium* spp. RBL 68 and 85 were purified from the culture supernatants by combination of methanol-acetone extraction, cation-exhange SP-Sepharose and Sep-Pack C18 cartridge (Tables 2 and 3).

As shown previously, the two BLIS from *Bifidobacterium* spp. RBL 68 and 85 supernatants were recovered by methanol-acetone extraction. Based on activity measurement (Table 2 and 3), 38 and 11% of the BLIS activities present in the cell-free supernatant were recovered after methanol-acetone extraction, respectively.

Several methods have been reported in the literature describing the purification of bacteriocins and their simulated compounds from bacterial culture supernatants. In the first step, the cation-exchange chromatography was used as a method of separation based on the interaction between bacteriocincations and resin-bound anionic groups (Guyonnet et al., 2000). Such method has been recently used to recover pediocin PA-1 produced by *Pediococcus acidilactici* UL5 at a yield of 8.3% from cell-free supernatant, providing a seven-fold increase in specific activity (Gaussier et al., 2002) . The use of sodium chloride (5% NaCl) to elute the two BLIS from cation-exhange SP-Sepharose column may have interfered with the proportion of lateral groups, and thereby reduced its antibacterial activity. The purification protocol resulted in a purification fold of 8.5 and 29 with a specific activity of 185 and 595AU mg^{-1} and a yield of 57 and 45% for the RBL 68 BLIS and RBL 85 respectively as shown in Tables 2 and 3. This latter step is based on cationic characteristic of studied substance and then we suggested that the two eluted BLIS may be cationic.

Final purification step of two BLIS consisted in loading on a Sep-Pak C18 column. This step showed the hydrophobic or amphiphilic nature of BLIS from *Bifidobacterium* spp. RBL 68 and 85. De-salting the two *Bifidobacterium* spp. RBL 68 and 85 BLIS containing solutions on a Sep-Pack C18 cartridge resulted in a 122.8 and 95-fold increase in specific activities with a yield of 85.7 and 34.3 respectively (Tables 2 and 3).

Partially purified BLIS from *Bifidobacterium* spp. RBL 68 and 85 had inhibitory activities towards species belonging to the same genus, towards LAB strains and Gram-positive bacteria including *Lactococcus, Enterococccus,* and *Streptococcus* . It is worth noting that this result is different from that of the inhibition data for bifidocin B (Yildirim et al. 1998). Bifidocin B is not able to inhibit *Streptococcus* and Gram-negative bacteria. The partial purified BLIS from *Bifidobacterium* spp. RBL 68 is not active towards species belonging to the following genus:
Lactobacillus, Bifidobacterium, and *Propionobactreium.*

In a similar fashion to the bacteriocin Bifidin I produced by *Bifidobacterium infantis* BCRC 14602 and BLIS produced by six *Bifidobacterium* strains (BIR-0304, BIR-0307, BIR -0312, BIR-0324, BIR-0326, and BIR-0349) that were active against Gram-positive and Gram-negative bacteria including *Salmonella, Shigella*, and *E. coli* (Cheikhyoussef et al., 2009, 2010; Collado et al., 2005), the two partial purified BLIS produced by RBL68 and RBL 85 were active against Gram-positive and Gram-negative bacteria. This result does not represent a common feature for the vast majority of bacteriocins from lactic acid bacteria (Van Belkum and Stiles, 2000). However, some exceptions with broad activity spectra described in recent years showed the ability to inhibit the growth of Gram positive and Gram-negative micro-organisms (Cheikhyoussef et al., 2009, 2010; Gao et al., 2010; Todorov and Dicks, 2005; Todorov et al., 2007;

De Kwaadsteniet et al., 2005; Kang and Lee, 2005). Interestingly, the strongest activity of the two BLIS was detected against some Gram-positive pathogens, especially *Listeria monocytogenes.* The anti-listerial activities displayed by strains RBL 68 and RBL 85 are characteristic of class IIa bacteriocins (Klaenhammer, 1993; Ennahar et al., 2000). *In vivo* studies demonstrated indeed that bacteriocin production improves the establishment success of the producing strains (Meghrous et al., 1990). The ability to synthesise bacteriocins is widely distributed among microbial collectivities of the gastrointestinal tract.

It has been reported that bacteriocins serve various functions in microbial communities (Klaenhammer, 1988) and may also play a defensive role and act to inhibit the invasion of other strains or species into an occupied niche or limit the advance of neighbouring cells (Riley and Wertz, 2002). Hence, the importance of the RBL68 and RBL 85 study, subject of this paper. These two BLIS strains were found to be proteinaceous compounds and exhibited robust activity against food-borne diseases and food spoilage pathogens such as *L. monocytogenes*, which make them potentially useful as antimicrobial agents in foods.

Conclusion

The production of the two RBL 68 and RBL 85 BLIS began in late exponential growth phase and reached a maximum activity during and after stationary phase. Their inhibitory activities were completely eliminated after treatment with proteinase for BLIS RBL 68 and -chymotrypsin, pronase E and proteinase K for BLIS RBL 85 suggesting that BLIS RBL 68 and RBL 85 are structurally different.

The two BLIS were partially purified from *Bifidobacterium* spp RBL 68 and RBL 85 by 3-step purification procedures resulting in a specific activity of 2.66×10^3 and 7.68×10^3 AU/mg and a purification fold of 122.8 and 95, respectively. Their inhibitory spectrum includes Gram-positive and Gram-negative bacteria; which is an important property of BLIS in food preservation. The broad spectrum of antimicrobial activities, and their resistance to pH and heat, makes the two partial purified BLIS good candidate as a natural fermented food preservative especially against food-borne Gram positive.

REFERENCES

Cheikhyoussef A, Pogori N, Chen HQ, Tian FW, Chen W, Tang J (2009). Antimicrobial activity and partial characterization of bacteriocin-like inhibitory substances (BLIS) produced by *Bifidobacterium infantis* BCRC 14602. Food Control, 20: 553–559.

Cheikhyoussef A, Cheikhyoussef N, Chen H, Zhao J, Tang J, Zhang H, Chen W (2010). Bifidin I – A new bacteriocin produced by *Bifidobacterium infantis* BCRC 14602: Purification and partial amino acid sequence. Food Control, 121: 746–753.

Collado MC, Hernandez M, Sanz Y (2005). Production of bacteriocin-like inhibitory compounds by human fecal *Bifidobacterium* strains. J. Food Prot., 68: 1034-1040.

De Kwaadsteniet M, Todorov SD, Knoetze H, Dicks LMT (2005). Characterization of a 3944 Da bacteriocin, produced by *Enterococcus mundtii* ST15 with activity against Gram-positive and Gram-negative bacteria. Int. J. Food., 105: 433-444.

De Vuyst L, Vandamme EJ (1994). Bacteriocins of lactic acid bacteria: Microbiology genetics and applications. London: Blackie.

Ennahar S, Sashihara T, Sonomoto K, Ishizaki A (2000). Class IIa bacteriocins: biosynthesis, structure and activity. FEMS Microbiol. Rev., 24: 85-106.

Gao Y, Jia S, Gao Q, Tan Z (2010). A novel bacteriocin with a broad inhibitory spectrum produced by *Lactobacillus sake* C2, isolated from traditional Chinese fermented cabbage. Food Control., 21: 76–81.

Gaussier H, Morency H, Lavoie M, Subirade M (2002). Replacement of trifluoroacetic acid with HC1 in the hydrophobic purification steps of pediocin PA1: a structural effect. Appl. Environ. Microbiol., 68: 4803-4808.

Gong HS, Meng XC, Wang H (2010). Plantaricin MG active against Gram negative bacteria produced by Lactobacillus plantarum KLDS1.0391 isolated from ‘‘Jiaoke”, a traditional fermented cream from China. Food Control, 21: 89–96.

Guyonnet D, Fremeaux C, Cenatiempo Y, Berjeaud JM (2000) Method for rapid purification of class IIa bacteriocins and comparison of their activities. Appl. Environ. Microbiol., 66: 1744-1748.

Ghrairi T, Frere J, Berjaud JM, Manai M (2005). Lactococcin MMT24, a novel two-peptide bacteriocin produced by *Lactococcus lactis* isolated from rigouta cheese. Int. J. Food Microbiol., 105: 389–398.

Holck A, Axelsson L, Schillinger U (1996). Divergicin 750, a novel bacteriocin produced by *Carnobacterium divergens*750. FEMS Microbiol. Lett., 1(36): 163-168.

Jack RW, Tagg JR, Ray B (1995). Bacteriocins of gram-positive bacteria Microbiol. Rev., 59: 171-200.

Kang JH, Lee MS (2005). Characterization of a bacteriocin produced by *Enterococcus faecium* GM-1 isolated from an infant. J. Appl. Microbiol., 98: 1169-1176.

Kaoutar B, Ilham M, Emna A, Aurelio S, Abdelaziz S (2010). Isolement and Purification of two Bacteriocins 3D Produced by *Enteorcoccus faecium* with inhibitory activity against *Listeria monocytogenes.* Curr. Microbiol., 62: 479-485.

Klaenhammer TR (1993). Genetics of bacteriocins produced by lactic acid bacteria FEMS Microbiol. Rev., 12: 39-85.

Klaenhammer TR (1988). Bacteriocins of lactic acid bacteria. Biochimie 70: 337–349.

Liliana M, Pascual M, Daniele B, Walter G, Maria C, Pajaro I, Barberis L (2008). Purification and Partial Characterization of Novel Bacteriocin L23 Produced by *Lactobacillus fermentum* 23. Curr. Microbiol., 56: 397-402.

Liu GR, Lv YN, Li PL, Zhou K, Zhang JL (2008). Pentocin 31-1, an anti-Listeria bacteriocin produced by *Lactobacillus pentosus* 31-1 isolated from Xuan-Wei Ham, a traditional China fermented meat product. Food Control, 19: 353-359.

Mathieu F, Michel M, Lefebvre G (1993). Properties of a bacteriocin produced by *Carnobacterium piscicola*CP5. Biotechnol. Lett., 15: 585-590.

Meghrous J, Lacroix C, Bouksaim M, Lapointe G, Simard R (1997). Genetic and biochemical characterization of nisin Z produced by *Lactococcus lactis* subsp. *lactis* biovar. diacetylactis UL719. J. Appl. Microbiol., 83: 133-138.

Meghrous J, Eulogy P, Junelles A, Ballongue J, Petitdemange H (1990) Screening of bifidobacteria strains for bacteriocin production. Biotechnol. Lett., 12: 575-580.

Pilet MF, Dousset X, Barre R, Novel G, Dcsmazeaud M, Piard JC (1995). Evidence for two bacteriocins produced by *Carnobacterium piscicola*and *Carnobacterium divergens* isolated from fish and active against *Listeria monocytogene.* J. Food Prot., 58: 256-262.

Riley MA, Wertz JE (2002). Bacteriocins: evolution, ecology, and application. Annu. Rev. Microbiol., 56: 117-137.

Schillinger U, Stiles ME, Holzapfel WH (1993). Bacteriocin production by *Carnobacterium piscicola* LV 61. Int. J. Food Microbiol., 20: 131-147.

Tagg JR, Adnan AS, Wanna-Maker LW (1976). Bacteriocins of gram-

positive bacteria. Bacteriol. Rev., 40: 722-756.
Todorov SD, Dicks LM (2005). *Lactobacillus plantarum* isolated from molasses produces bacteriocins active against Gram-negative bacteria. Enzyme Microb. Tech., 36: 318-326.
Todorov SD, Nyati H, Meincken M, Dicks LMT (2007). Partial characterization of bacteriocin AMA-K, produced by *Lactobacillus plantarum* AMA-K isolated from naturally fermented milk from Zimbabwe. Food Control, 18: 656-664.
Toure R, Kheadr E, Lacroix C, Moronio FI (2003). Production of antibacterial substances by bifidobacterial isolates from infant stool active against *Listeria Monocytogenes.* J. Appl. Microbiol., 95: 1058-1069.
Van Belkum MJ, Stiles ME (2000). Nonlantibiotic antibacterial peptides from lactic acid bacteria. Nat. Prod. Rep., 17: 323-335.
Yildirim Z, Winters DK, Johnson MG (1999). Purification, amino acid sequence and mode of action of bifidocin B produced by *Bifidobacterium bifdum* NCFB 1454. J. Appl. Microbiol., 86: 45-54.
Yildirim Z, Johnson MG (1998). Characterization and antimicrobial spectrum of bifidocin B, a bacteriocin produced by *Bifidobacterium bifidum* NCFB 1454. J. Food Protect., 61: 47-51.