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Full Length Research Paper

Antibacterial activity and probiotic properties of some lactic acid bacteria isolated from dairy products

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Several lactic acid bacteria strains were screened for the production of antibacterial substances active against some pathogenic bacteria. The inhibitory mechanism was investigated and was shown to be dependant of bacteriocin production. The objective was to isolate LAB with antibacterial activity from raib and to select strains that could be used for the determination of probiotic properties. Results showed that 6 LAB cultures retained activity in the supernatants after neutralization and catalase treatment. *Streptococcus thermophilus* T2 strain showed the wide inhibitory spectrum against the Gram positive bacteria. Growth and bacteriocin production profiles showed that the maximal bacteriocin production by *S. thermophilus* T2 cells was measured by the end of the late-log phase (90 AU ml^{-1}) with a bacteriocin production rate of 9.3 (AU ml^{-1} h^{-1}). In addition, our findings showed that the bacteriocin, produced by *S. thermophilus* T2, was stable over a wide pH range (4 - 8), indicating that such bacteriocin may be useful in acidic as well as non acidic food. This preliminarily work shows the potential application of autochthonous lactic acid bacteria to improve safety of traditional fermented food. Around 40% of these strains, mainly isolated from Algerian fermented milks in MRS agar, were pre-selected for testing as potential probiotics by their ability to grow adequately at low pH values. *In vivo* study indicates that number of *Sc T2* and *Lb. B2* remains important as long as rabbits consume some fermented products. Antibacterial activity of Lactobacillus *and Streptococcus against* indicator bacteria is a good probiotic property. Transfer of these antimicrobial activities to *in vivo* conditions is beneficial for the maintenance of the intestinal microflora.

Key words: Bacteriocin, lactic acid bacteria, *Streptococcus thermophilus*. *Lactobacillus bulgaricu,* probiotic.

INTRODUCTION

Thirteen (13) lactic acid bacteria (LAB) isolated from dairy products have received increased attention as a potential food-preservative due to their antagonistic activity against many food born pathogen such as *Listeria monocytogenes* (Mataragas et al., 2002; Jamuna and Jeevartnam, 2004). LABs are widely distributed in nature; they are typically involved in a large number of spontaneous food fermentation and have been extensively studied (Holzapfell et al., 1995). Some members of LAB produce bacteriocins and bacteriocins- like substances which may inhibit growth of spoilage and pathogenic microorganisms (Klaenhammer, 1988). Bacteriocins from LAB are unit; bioactive peptides or proteins with antimicrobial activity towards gram positive bacteria, including closely related strains or spoilage and pathogenic bacteria (Tagg et al., 1976). Bacteriocins are ribosomally synthesized and extracellularily released bioactive peptides or peptide-complexes which have bactericidal or bacteriostatic effect (Garneau et al., 2002). Use of either their bacteriocins or the bacteriocin-producing LAB like starter cultures for food preservation has received a special attention (Sabia et al., 2002). Moreover, bacteriocins are innocuous due to proteolytic degradation in the gastrointestinal tract (Vuyst and Vandammemmm, 1994).

Streptococcus thermophilus is a lactic acid bacterium of major importance for the food industry as regard the manufacture of yoghourt (Purwandari et al., 2007). Some of *S. thermophilus* strains produce a bacteriocin named thermophilin (Marciset et al., 1997) which is active against several LAB and food spoilage bacteria such as *Clostridium*

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Abbreviations: LAB, Lactic acid bacteria; **CFU,** colony forming **HPLC,** high performance liquid chromatography.

sporogenes. In view of its technological and biochemical properties, the above bacteriocin can be considered as a potential biopreservative (Aktypis et al., 2007). Many types of LAB have been used as probiotics since time immemorial (Lactobacilli, Streptococci, Enterococci and Lactococci; Bifidobacteria and Bacillus species). A potential probiotic organism must possess the following attributes: ability to survive the condition in the gut, antimicrobial production and ability to adhere to the intestinal cell of the host (Mataragas et al., 2003). Probiotics are organisms which are introduced orally in the gastrointestinal (GI) tract, where they are expected to contribute positively to the activity of the intestinal micro biota and thus, to the health of the host (Saarela et al., 2002). In order to exert such an activity, they have to compete with the autochthonous micro flora. When selecting new probiotic strains or testing functional properties of the existing ones, the screening of adhesion properties is considered an important step. In the present study, the inhibitory effect of the cell-free filtrates of each of the 13 isolates was evaluated.

MATERIALS AND METHODS

Isolation of lactic acid bacteria

The bacterial strains used in this study were isolated from fermented traditional milk Raib, manufactured without starter cultures. Raib is made from the raw cow milk; milk fermentation is spontaneous and uncontrolled and could be a valuable source of autochthonous lactic acid bacteria (LAB). About 30 samples were collected all over chef regions and obtained with collaboration of Bioresources Research Laboratory. LAB was isolated from Raib, by homogenizing 10 g samples of cheese in 90 ml saline solution and then plating suitable serial dilutions onto different media: BHI, MRS and M-17 (Biokar diagnostics, Beauvais, France). The plates were incubated aerobically at 30°C for 48 h and then several colonies were picked at random for identification. Cell morphology and gram-staining reaction were examined by light microscopy and the catalase activity was carried out. Phenotypic identification was based upon physiological and biochemical characteristics, 22 reactions (sugar fermentation) were determined and some of them provided a means of discriminating them. Sugar fermentation profile, in the API - 20 Strep CH and API - 50 CH fermentation, was carried out according to the manufacturer's instructions (Bio Merieux, Marcy l'Etoile, France).

Detection of antibacterial activity

For detection of antagonistic activity, an agar spot test was used. The agar spot test was a modification of that described by Tomé et al. (2006). Overnight cultures, on MRS medium, of the strains to be tested for production of antimicrobial compound were centrifuged (10 min at 15000 *g*, 4°C). Cell-free supernatants were filtered across cellulose acetate filter (0.2 µm) to remove residual cells.

An overnight (37°C) of the target strain was diluted in sterile Mueller Hinton medium and 2 ml of this dilute culture was spread on solid Muller Hinton medium. After 5 min of contact the excess was removed and the Petri dishes were dried for 10 min. Samples (10 µl) of filtered cell-free supernatants were spotted on the agar plate. The target strains used in this study are *Bacillus cereus* CIP 6624*, Bacillus subtilis* ATCC 6633*, Escherichia coli* CIP 35218, *Enterococcus feacalis* CIP 29212*, Listeria innocua* ATCC 51742*, Salmonella typhimurium* CIP 5858*, Staphylococcus aureus* CIP 29213*, Staphylococcus epidermitidis* ATCC 14990. The antagonistic activity was evaluated finely by measurement of clear zones around spots of the putative producers.

Sensitivity of bacteriocin to enzymes, pH and heat treatment

The biochemical nature of the antibacterial agent was studied on both chloroform extract and cell-free supernatant; all the samples were incubated for 1 h at 37°C before the antibacterial essay. The pH of cell-free supernatants was adjusted to 6.5 with NaOH (1 N). Catalase (Sigma, 500 IU ml^{-1}) activity was tested by spotting colonies with 3% hydrogen peroxide. The cell-free supernatant was also submitted to heat treatment (60 - 95°C for 30 min) and to several pH (4 - 8). The chloroform extract was treated with α-amylase (Sigma, 1 mg ml^{-1} 100 mM phosphate buffer, pH 6.9), α-chymotrypsin (Sigma, 1mg ml^{-1}, 0.05 M Tris–HCl buffer (pH 8.0)-0.01 M $CaCl_2$), Pronase E (Sigma, 1mg ml^{-1} in 100 mM Tris–HCl buffer, pH3), Proteinase K (Sigma, 1 mg ml^{-1} in 100 mM Tris–HCl buffer, pH 7.5) and Trypsin (Sigma, 1 mg ml^{-1} 50 mM Tris–HCl buffer pH 8.0). Prior to being assayed for bacteriocin activity, preparations containing pronase E were adjusted to pH 6.0. Neutralized cell-free supernatant neutralized cell-free supernatant treated with catalase; heat-treated supernatant and chloroform-extract were spotted against *L. innocua*. The enzymes were heat-inactivated for 3 min at 100°C. For each test, untreated bacteriocin plus buffer; bacteriocin plus buffer treated for 5 min at 100°C; buffer alone and enzymes solutions served as controls (Cherif et al., 2003; Lyon and Glatz, 1991). Samples with and without enzymes were held at 37°C for 3 h and the remaining activity was determined by well diffusion assay as described before using *L. innocua as* indicator strains.

Growth kinetic and bacteriocin production

Growth experiments were performed in ErlenMeyer flask of 500 ml containing 250 ml of MRS broth (pH 6.5) at 37°C without shaking. An overnight pre-culture of *S. thermophilus* was used for the inoculation of the MRS broth at initial cell density of *ca.*10^3 CFU ml^{-1}. At different time intervals, samples were removed from the culture and used for optical density measurement (660 nm), viable and cultivable count (CFU ml^{-1}), extracellular pH measurements and bacteriocin production. The antibacterial concentration of each sample was conducted with the critical method of dilutions (Mayr-Harting et al., 1972). The bacteriocin concentration Arbitrary Unit ml^{-l} (AU ml^{-l}) was calculated as the inverse of the strongest dilution which induces the inhibition of *L. innocua*.

Bacteriocin extraction

The extraction was realized from cell-free culture supernatant of *S. thermophilus* obtained after centrifugation of overnight culture (20 min at 15000 g at 4°C). The extraction was performed according to Burianek and Yousef (2000). The culture supernatant (100 ml) was stirred vigorously for 20 min with chloroform (v/v) and transfered in separation funnel. The interface layer between the aqueous and organic phases, which contain bacteriocin, was harvested and the residual chloroform was eliminated by speed vacuum (50 H, Unique, Martinsried, Germany). Then bacteriocin was recuperated in the interface layer, aqueous and organic phases

HPLC purification of supernatant chloroform extract

The conditions for bacteriocin isolation were realized through analytical

Table 1. Lactic acid bacteria isolated from traditional dairy product.

Strain	Source	Growth medium
Lactococcus lactis S1	Raib	MRS
Lactococcus lactis S2	Raib	MRS
Lactococcus lactis S3	Raib	MRS
Lactococcus lactis S4	Raib	MRS
Lactococcus lactis S5	Raib	MRS
Lactococcus Lactis S6	Raib	MRS
Streptococcus thermophilus T1	Raib	MRS
Streptococcus thermophilus T2	Raib	MRS
Streptococcus cremoris R1	Raib	MRS
Streptococcus cremoris R2	Raib	MRS
Streptococcus cremoris R3	Raib	MRS
Lactobacillus bulgaricus B1	Raib	MRS
Lactobacillus bulgaricus B2	Raib	MRS

Table 2. Antibacterial spectrum of the cell-free supernatant of the six lactic acid bacteria isolated from the traditional dairy product (Raib) "A zone of inhibition being at least 2 mm must be observed".

Strain	Strains inhibited
Lctococcus lactis S1	*Listeria innocua*, *Enterococcus faecalis*
Lactococcus lactis S2	*Listeria innocua*
Lactobacillus bulgaricus B1	*Listeria innocua, Enterococcus faecalis Bacillus cereus Bacillus subtilis*
Lactobacillus bulgaricus B2	*Listeria innocua, Bacillus cereus*
Streptococcusthermophilus T2	*Bacillus cereus, Bacillus subtilis, Listeria innocua, Enterococcus faecalis, epidermitidis, Staphylococcus epidermitidis*
Streptococcusthermophilus T1	*Enterococcus faecalis, Bacillus cereus, Enterococcus faecalis*

RP-HPLC on the chloroform extract. The liquid chromatographic system consisted of a Waters 600E automated. gradient controller pump module, a Waters Wisp 717 automatic sampling device and a Waters 996 photodiode array detector. Spectral and chromatographic data were stored on a NEC image 466 computer. Millennium software was used to plot, acquire and analyze chromatographic data.

All of the chromatographic processes were performed on an Uptisphere C_{18} column (150 × 4.6 mm, UP5ODB615QS, Interchim, Montluçon, France). The mobile phase was water/ trifluoroacetic acid (1000:1, v/v) as eluent A and acetonitrile/ trifluoroacetic acid (1000:1, v/v) as eluent B. The flow rate was 1 ml min^{-1}. Samples were filtered through 0.22 mm filters and then injected. The gradient applied was 0 - 50% (v/v) B over 100 min, then 50 - 100% (v/v) B over 5 and 15 min at 100% (v/v) B. On-line UV absorbance scans were performed between 200 and 300 nm at a rate of one spectrum per second with a resolution of 1.2 nm. Chromatographic analyses were completed with millennium software (Zhao et al., 1997).

Animal model (rabbits)

For this experiment we have chosen 12 rabbits distributed in three lots as laboratory animal. Rabbits (Oryctolagus cuniculus) have same age (5 months), weighing between 1000 and 1100 g at the beginning of the experiment. Animals are put individually in metallic cages of 50 cm side, are maintained in a well aerated pet shop, in constant temperature of 21 ± 1°C with a lighting of 12 H i. The food and the water are distributed *ad libitum* and the cleaning of cages is every morning insured.

Diet of rabbits

We have used 12 rabbits which are fed at first with a standard diet (250 g a day of lettuce, carrots, bread and water) during a period of adaptation of 7 days. Then and during the next 4 days, the diet of rabbits is supplemented by 2 × 10 ml a day of dairy products fermented by LAB strains hours. This supplementation is stopped within 4 days. Fecal samples were collected and homogenized in a peptone-saline solution. To estimate the concentration of lactic acid bacteria, appropriate dilutions were spread in quadruplicate onto plates of MRS agar. The cultures were incubated in anaerobiosis at 37°C for 24 h. From the fecal samples of each volunteer, 50 colonies grown in MRS agar were randomly selected and checked for every property.

RESULTS

Antimicrobial activity

13 LAB strains, isolated from Algerian dairy milk (Table 1), were screened for their antagonistic activity against *L. innocua*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus subtilis, Sc. aureus, Staphylococcus epidermidis*, *E. coli* and *S. typhimurium*. The results of Table 2 show that six

Table 3. Effect of different treatments on cell-free supernatant and chloroform extract of *Sc. thermophilus* T2.

Treatment	Relative activity
Enzymatic treatment	
Proteinase K	–
Pronase E	–
α-chymotrypsin	–
Trypsin	–
α-amylase	++
Catalase	++
Control	+++
pH treatment	
4	+++
5	+++
6	+++
7	+++
8	+++
Control	+++
Heat treatment (°C)	
60	+++
70	+++
80	++
90	++
95	+
Control	+++

Relative activity was measured by an agar diffusion test against *L. innocua*. (–): No inhibition, (+): slight inhibition, (++): moderate inhibition, (+++): strong inhibition.

isolates were active against one or more tested strains. However, *S. thermophilus* T2 strain showed a wide inhibitory spectrum against all the gram positive target bacteria used in this study except against *S. aureus* (Table 2). In addition, *S. thermophilus* T2 did not show any inhibitory activity against the gram negative bacteria (*E. coli* and *S. typhimurium)* used in this study.

Nature of the inhibitory agent

Our results showed that the free-cell supernatant remained active against sensitive target strains, even when the pH was adjusted to 7. However, when the cell-free supernatant and the chloroform-extract were exposed to the proteolytic enzymes (Table 3), no inhibitory activity was observed against *L. innocua* in contrast to the control tests which showed an inhibitory activity against the target strain (Table 3). In addition, when the cell-free supernatant and the chloroform-extract were exposed to the action of α-amylase and catalase, similar inhibitory activity was measured when compared to the control against *L. innocua*. These results suggest that the biochemical nature of the molecule produced is peptidic. Moreover, the antimicrobial activity appeared to be heat resistant. The inhibitory activity of the chloroform-extract was still measured after 30 min heat treatment at 90°C. Our results showed also that in range of pH 4 - 8 similar antibacterial activities of the chloroform extract were obtained against *L. innocua*.

Extraction of the bacteriocin produced by *S. thermophilus*

The extraction of the bacteriocin produced by *S. thermophilus* T2 strain from culture supernatant was realized with chloroform, a water-immiscible solvent. The method used concentrated the bacteriocin at the interface between chloroform and the aqueous culture of the producing bacterium. In addition, the precipitate at the interface between the chloroform and culture supernatant fluid contained most of the bacteriocin activity in the mixture. The precipitate at the interface was harvested and the residual chloroform was eliminated by speed vacuum. After HPLC reversed-phase chromatography, bacteriocin activity was associated with two peaks eluting at 17 and 110 min (Figure 1). These results showed that the antibacterial activity of *S. thermophilus* T2 could be associated with two molecules which present different hydrophobicity.

Growth kinetics and bacteriocin biosynthesis

Growth and bacteriocin production of *S. thermophilus* was studied in MRS broth at 37°C at pH of 6.5 in standard plate count. Under these conditions bacteriocin activity was detected after 4 h of incubation at the beginning of the exponential phase, at a cell concentration of 10^4 CFU ml^{-1} (12 AU ml^{-1}). The results of Figure 1 showed that bacteriocin production increases with the increase of cell concentration to reach a maximum of 90 AU ml^{-1} with a bacteriocine production rate of 9.3 (AU ml^{-1}) h^{-1}. This concentration was reached between 12 and 14 h of incubation at 37°C. During the stationary phase both bacteriocin concentration and the cell concentration remained at a steady state (Figure 1). Antibacterial activity decreased after 24 h of incubation, having reached maximum levels after 14 h (data not shown).

Survivability in gastro-intestinal

Survival of Streptococcus T2 and Lactobacillus.B2

The rate of lactic bacteria in the duodenum and the stomach of rabbits are shown in Figures 3 and 4. Although these manage to surmount the drastic conditions of the digestive tract, their rates remain relatively stable during all the period of supplementation. The acidity reigning in the

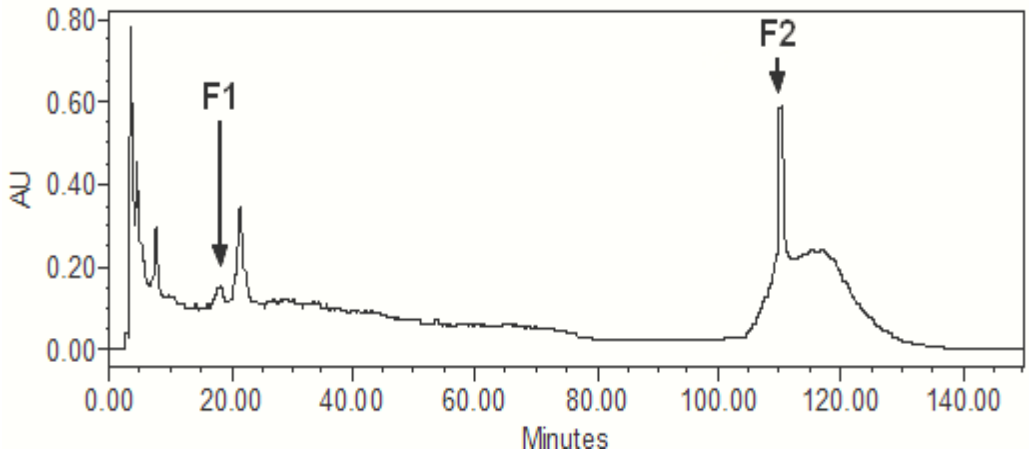

Figure 1. Elution pattern of chloroform-extract from *Sc. thermophilus* T2 strain by reversed-phase high-performance liquid chromatography.

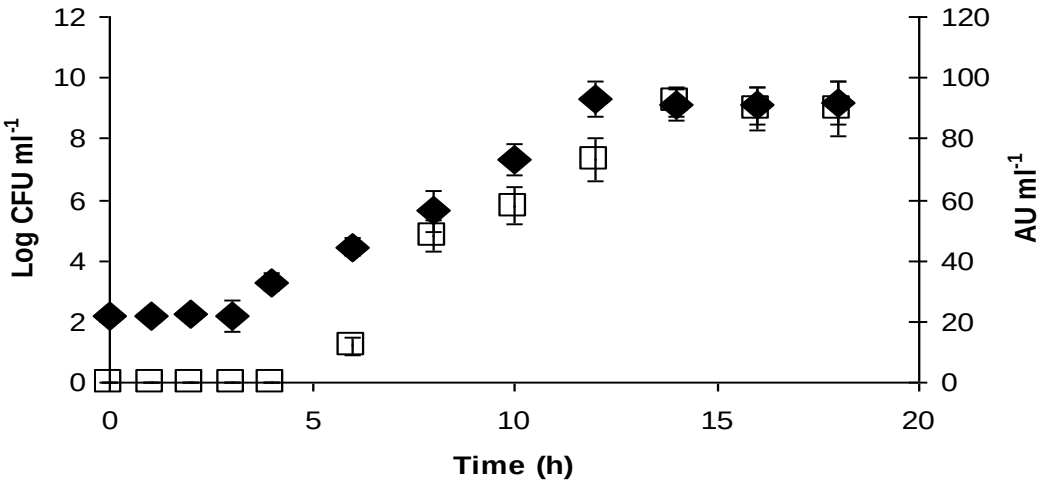

Figure 2. Growth kinetic and bacteriocin production by *Sc. thermophilus*. The growth was performed at an initial pH of 6.5, at 37°C without shaking. (◆) growth kinetic, (□) bacteriocin production. The experiments were repeated three times and results represent the mean ± standard error of the mean.

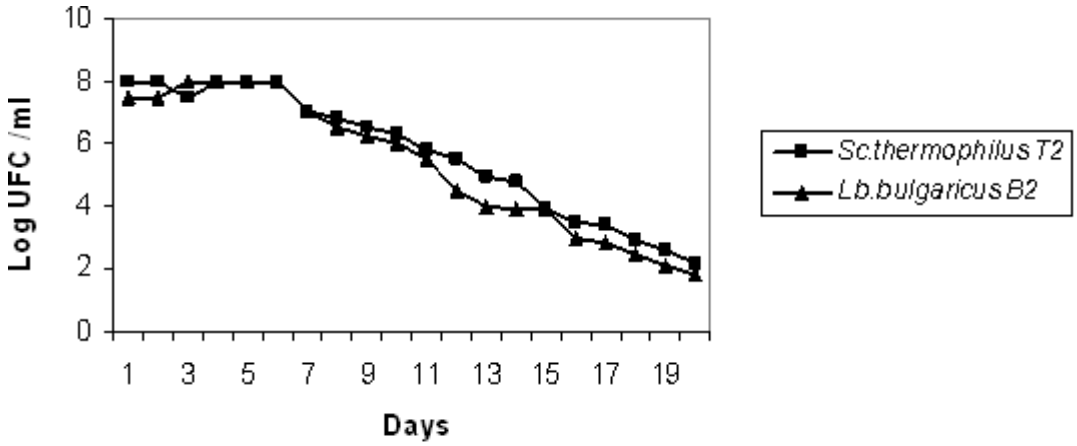

Figure 3. Progress of LAB strains in the stomach of rabbits.

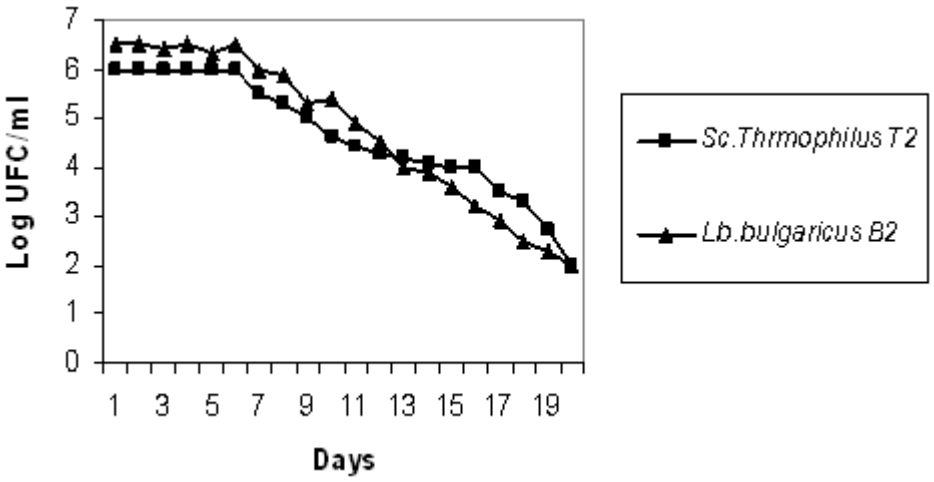

Figure 4. Progress of LAB strains in the duodenum of rabbits.

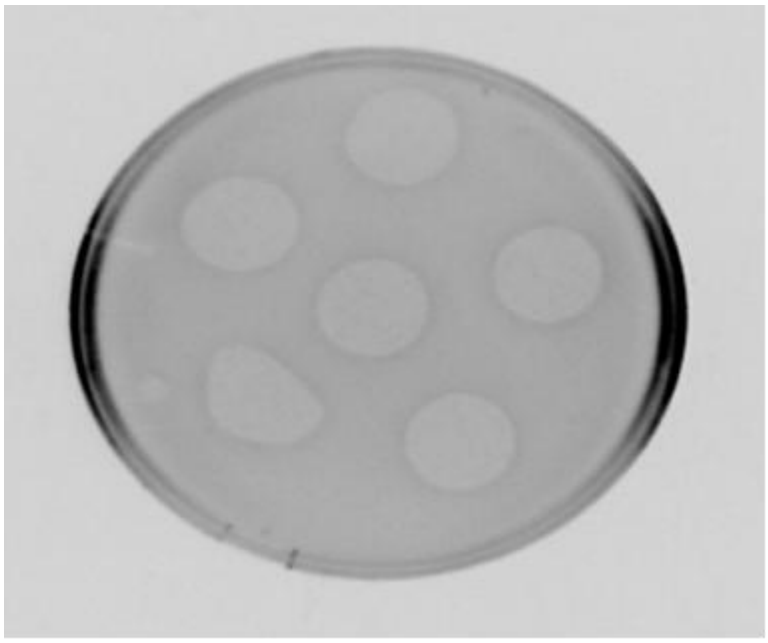

Figure 5. Antagonistic agar test showing the antibacterial activity of *Sc. thermophilus* T2 during passage through stomach of rabbits.

stomach represents destroying physiological barrier for bacteria and enzymes naturally present in the food. However, some lactic fermentation resists in this aggressive barrier. Enumeration on Petri dish revealed the presence of *Sc*. T2 and LbB2, their concentration remains important, and superior to 10^8 cellules during all the period of supplementation.

Activity assay

The isolated strains during digestion were screened for their antagonistic activity against *L. innocua* using the antagonistic test as described above. The isolates were inoculated into broth media (MRS1 or M17m) and incubated for 24 h at 30°C. Culture supernatant was prepared and assayed for the presence of an inhibitor in the broth following the agar well diffusion assay (Barefoot and Klaenhammer, 1983a). Inhibition of growth was determined by an area of inhibition surrounding each agar well. It appears from Figure 5 that the passage through the gastro-intestinal tract did not affect the inhibitory effect of *S. thermophilus T2.* Also, it was evident that the inhibitory did not attribute to acid production by *S. thermophilus*

since the MRS was supplemented with 0.2% (w/v) sodium bicarbonate to neutralize acidity. The production of inhibitory zones by tested LAB strains revealed that the passage through the gastro-intestinal tract did not affect bacteriocin production.

DISCUSSION

Recently, the use of probiotic strains (particularly lactobacilli and bifidobacteria) has been promoted as a means to balance the gut microbiota and in fact, their potential preventive and therapeutical effects have received renewed research and industrial interest (Salminen et al., 1998; Ouwehand et al., 1999; Saavedra, 2001).

Bacteriocins from lactic acid bacteria are of importance in bioconservation of various foods. Moreover, the use of more than one LAB bacteriocin as a combination of biopreservative may have major applications in improving food safety (Jamuna and Jeevartnam, 2004) and only against gram positive bacteria. The biochemical nature of the antibacterial molecule produced by *S. thermophilus* T2 was studied for both the cell-free supernatant and the chloroform-extract. Our results showed that the molecule, produced by *S. thermophilus*, is peptidic since the antibacterial activity of the molecule was lost after digestion with proteolytic enzymes. However, the neutralization (pH 7) and addition of catalase of α-amylase to the cell-free supernatant did not result in the loss of the antilisteral activity. Our results also showed that the bacteriocin produced by *S. thermophilus* is heat stable (up to 30 min at 95°C); these results are similar to what has been reported for thoenicin (Merwe et al., 2003). In addition, the bacteriocin was stable over a wide pH range, indicating that such bacteriocin may be useful in acidic as well as non acidic food. Similar pH stability results have been reported for propionicin PLG1 (Lyon and Glatz, 1991). Growth and bacteriocin production profiles showed that the maximal bacteriocin production was measured by the end of the late-log phase. The level of production remained at a steady state during the stationary phase. Similar results were obtained by Ivanova et al. (1998). However, bacteriocin production decreased after 24 h of incubation, having reached maximum levels after 14 h. This reduction could be a result of the inactivation of bacteriocin by extracellular proteases. Preliminarily characterization of the bacteriocin produced by *S. thermophilus* T2 was realized in the present study. It was found that the bacteriocin inhibits closely related gram positive strains like *L. innocua* and *E. faecalis*. Activity against gram negative was rarely reported for bacteriocin (Martinez-bueno et al., 1990; Turgeon and Moineau, 1991). Active substance from culture supernatant of *S. thermophilus* T2 was obtained according to the procedure described by Burianek and Yousef (2000).

The administration of 2 × 10 ml a day of dairy fermented milk in rabbits leads to the presence of high concentrations of *S. T2* and of *Lb. B1* in the stomach, in the duodenum. The pH plug has certainly allowed both fermentation to resist the acidity of the gastric juice, to the biliary salts, during all the period of the ingestion of fermented products (7 days) and with a considerable number of bacterial cells. The number of Sc.T2 and *Lb. B2* remains important as long as rabbits consume some fermented products. The results are in perfect concordance with those of Dilmibouras and Sadoun (2002). Number of survival bacteria begins to decrease gradually till it disappeared completely after a fortnight that followed the stop of supplementation. These results are of a big interest for possible uses of these strains in therapeutic or metabolic purposes (reduction of the excessive cholesterol in the blood, the hydrolysis of the lactose and in infection intestinal (Dilmi-Bouras and Sadoun, 2002). Resistance to low pH and bile fluid is usually considered as good indicators for survival of bacterial strains through the GI (gastrointestinal) tract and as required properties of a probiotic strain. The criteria for selecting a good probiotic strain have been listed comprehensively by several authors. The strains should possess a generally regarded as safe (GRAS) status and be able to survive through the gastrointestinal tract (ghelfi et al., 2005). *In vitro* inhibitory activity of *Lactobacillus and Streptococcus* against indicator bacteria is a good probiotic property. Salminen et al. (1998) stated that if antimicrobial activities could be transferable to *in vivo* conditions, it seems beneficial for the maintenance of the intestinal microflora. The survival of ingested probiotics at different levels of the gastrointestinal tract was measured *in vivo* using intestinal intobations, but other techniques can be used for identification of strain on mucosal biopsies. Several *in vitro* models can help to predict the fate of ingested strains.

In conclusion, study of autochthonous LAB will permit us to select the best candidates for improving the microbiological safety of traditional food products such as Raib and may increase their shelf life. Such a collection could be used for construction of specific starter cultures with probiotics properties. Indeed few studies have been focused on the effects of probiotics on the intestinal function of healthy people and the observed effects depend on the strain used (Oliwar et al., 2005).

REFERENCES

Aktypis A, Tychowski M, Kalantzopoulos G, Aggelis G (2007). Studies on bacteriocin (thermophilin T) production by *Streptococcus thermophilus* ACA-DC 0040 in batch and fed-batch fermentation modes. *Antoine van Leeuwenhoek* (92): 207-220.

Barefoot SF, Klaenhammer TR (1983a). Detectionanbactivity of lacticin B, a bacteriocin produced byLactobacillus acidophilus. Appl. Environ. Microbiol. 45: 1808-1815.

Burianek LL, Yousef AL (2000). Solvent extraction of bacteriocins from liquid cultures. Letters in Appl. Microbiol. 31: 193-197.

Cherif A, Rezgui W, Raddadi N, Daffonchio D, Boudabous A (2008). Characterization and partial purification of entomocin110, a newly identified bacteriocin from Bacillus thuringiensis subsp. Entomocidus HD110. Microbiol. Res. 163: 684-692.

De Vuyst L, Vandamme EJ (1994). Nisin, a lantibiotic produced by Lactococcus lactis subsp. Lactis: properties, biosynthesis, fermen-

tation and application. *In*: *Bacteriocins of lactic acid bacteria*. De Vuyst L and Vandamme EJ, (eds). Balackis Academic and Professional, Glasgow, pp. 151-221.
Dilmi-Bouras A, Sadoun D (2002). Survie des ferments du yaourt dans le tube digestif du lapin Lait. 82: 47-253
Garneau S, Martin NI, Vederas JC (2002). Two peptide bacteriocins produced by lactic acid bacteria. Biochimie, 84: 577-592.
Ghelfi F, Verdenelli MC, Silvi S, Orpianesi C, Cresci A (2005). Probiotic properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faeces. Euprobio, 6: p. 8.
Holzapfel WH, Geisen R, Schillinge U (1995). Biological preservation of foods with reference to protective cultures, bacteriocins, and food-grade enzymes. Int. J. Food Microbiol. 24: 343-362.
Ivanova I, Miteva V, Stefanova T, Pantev A, Budakov I, Danova S, Moncheva P, Nikolova I, Dousset X, Boyaval P (1998). Characterization of a bacteriocin produced by *Streptococcus thermophilus* 81. Int. J. Food Microbiol. 42: 7-158.
Jamuna M, Jeevaratnam K (2004). Isolation and partial characterisation of bacteriocins from *Pediococcus* species. Appl. Microbiol. Biotechnol. 65(4): 433-439.
Klaenhammer TR (1988). Bacteriocin of Lactic Acid Bacteria. Biochimie, 70: 337-349.
Lyon W, Glatz BA (1991). Partial purification and characterization of a bacteriocin produced by *Propionibacterium thoenii*. Appl. Environ. Microbiol. 57: 593-609.
Marciset O, Jeronimus-Stratingh C, Mollet B, Poolman B (1997). Thermophilin 13, a Nontypical Antilisterial Poration Complex Bacteriocin, That Functions without a Receptor J. Biol. Chem. 272(22): 14277-14284.
Martinez-bueno, Galvez A, Valdivia E, Maqueda M (1990). A transferable plasmid associated with AS48 production in *Enterococcus feacalis*. J. Bacteriol. (172): 2817-2818.
Mataragas M, Drosinos E.H, Metaxopoulos J (2003). Antagonistic activity of Lactic acid bacteria against *Listeria monocytogenes* in sliced cooked cured pork shoulder stored under vacuum or modified atmosphere at 4±2°C. Food Microbiol. 20: 259-256.
Harting M, Herdgs AJ, Berkeley RCW (1972). Methods for styding bacteriocins. Methods Microbiol. 7: 315-422.
Ouwehand AC, Kirjavainen PV, Shortt C, Salminen S (1999). Probiotics: mechanisms and established effects. Int. Dairy J. 9: 43-52.
Purwandari U, Shah NP, Vasiljevic T (2007). Effects of exopolysaccharide-producing strains of *Streptococcus thermophilus* on technological and rheological properties of set-type yoghurt. Int. Dairy J. 17: 1344-1352
Saarela M, Lähtenmäki L, Crittenden R, Salminen S, Mattila-Sandholm T (2002). Int. J. Food Microbiol. 78: 99-117.
Saavedra JM (2001). Clinical applications of probiotic agents. Am. J. Clin. Nutr. 73: 1147-1151.
Sabia C, Manicardi G, Messi P, De Niederhäusern, S, Bondi M (2002). Enterocin 416K1, an antilisterial bacteriocin produced by *Enterococcus casseliflavus* IM 416K1 isolated from Italian sausages. Int. J. Food Microbiol. 75: 163-170.
Salminen S, Deighton M.A, Benno Y, Gorbach SL (1998). Lactic acidbacteria in health and disease. In: Salminen S, von Wright (Eds.), LacticAcid Bacteria: Microbiology and Functional Aspects, (2nd edn) Marcel Dekker, New York.
Tagg JR, Dajani AS, Wannamaker LW (1976). Bacteriocins of gram positive bacteria. Bacteriol. Rev. 40: 722-756.
Tomé E, Teixeira, Gibbs PA (2006). Anti-listerial inhibitory lactic acid bacteria isolated from commercial cold smoked salmon. Food Microbiol. 23: 399-405.
Zhao Q, Molina Piot JM (1997). Peptic peptide mapping by HPLC, on line with photodiode array detection, of a hemoglobin hydrolysate produced at pilot-plant scale from an ultrafiltration process. J. Liquid Chromatog. Related Technol. 20: 1717-1739.