International Journal of Animal and Veterinary Advances 5(2): 87-97, 2013
DOI:10.19026/ijava.5.5582
ISSN: 2041-2894; e-ISSN: 2041-2908

Submitted: February 06, 2013 Accepted: March 08, 2013 Published: April 20, 2013

Research Article

Effects of Inclusion of Two Probiotic Strains Isolated From "Sha'a", a Maize-Based Traditionally Fermented Beverage on Lipid Metabolism of Rabbits fed a Cholesterol-Enriched Diet

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Abstract: The objective of this study was to assess the *in vitro* probiotic potential of *Lactobacillus plantarum* Lp10S and Lp11S isolated from Sha'a and evaluate their effects on lipid metabolism of hypercholesterolaemic rabbits. The strains were tested *in vitro* for their acid and bile tolerance, capability to remove cholesterol from MRS broth and to deconjugate bile salts. For *in vivo* studies, *Lactobacillus plantarum* Lp10S and Lp11S were administered to rabbits fed on cholesterol-enriched diet (standard diet+2×0.02 g of pure cholesterol per day per rabbit) at a dose of 2×10^8 CFU per day per rabbit for each strain; the treatment lasted seven weeks and one week post ingestion period was observed. Serum lipids were analyzed during the experiment. The results revealed that strains *Lactobacillus plantarum* Lp10S and Lp11S tolerated well low pH and bile salts, expressed bile salt hydrolase activity and have ability to assimilate cholesterol *in vitro*. The results also showed that diets including *Lactobacillus plantarum* Lp10S and Lp11S significantly lowered serum total cholesterol, (VLDL+IDL+LDL)-cholesterol and triglycerides levels of hypercholesterolaemic rabbits. In addition, HDL-cholesterol level significantly increased and the atherosclerosis index was significantly lowered. The present results indicate the probiotic potential of *Lactobacillus plantarum* Lp10S and Lp11S strains as well as their *in vivo* cholesterol-lowering properties that may be useful for their application as health-promoting bacteria.

Keywords: Cholesterol-lowering effect, *Lactobacillus plantarum*, Probiotics, rabbits, Sha'a,

INTRODUCTION

Elevated serum cholesterol is associated with the development of diseases like cardiovascular hypercholesterolemia and atherosclerosis which are some of the most important causes of mortality both in developed and developing countries. Low levels of high-density lipoprotein cholesterol (HDL-C) and high levels of low-density lipoprotein cholesterol (LDL-C) are important risk factors. Published works indicate that the reduction of excessive cholesterol levels in the blood decreases the risk of these diseases (Buck and Gilliland, 1994). Given the increased risk of atherosclerosis with rising blood cholesterol levels, inhibitors of key enzymes involved in cholesterol biosynthesis are widely used to treat atherosclerosis. However, this current drug therapy has disadvantages in that it is costly and it has negative side effects. For instance, although statins (Pravastatin, Lovastatin, Simvastatin, Fluvastatin, Atorvastatin, Cerivastatin) reduce cholesterol levels in hypercholesterolemic patient by inhibiting the activity of β-hydroxy-β-methyl-glutaryl coenzyme A (HMG-CoA) reductase, they also inhibit the synthesis of coenzyme Q which is involved in various metabolic processes including electron transfer in ATP synthesis in mitochondrial respiration chain (Patrick and Uzick, 2001; Lankin *et al*., 2003). Therefore, there is an increasing interest in alternative therapies to lower blood cholesterol level with fewer side effects.

The search for novel probiotic strains is becoming of utmost importance in order to satisfy the increasing market demand for health foods. These products must contain probiotic cultures with novel properties, which can be screened from different origins or ecological niches, especially in areas or substrates where the natural microbiota have not yet been characterized.

It is against this backdrop that the present study was conducted to evaluate the *in vitro* probiotic

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potential (acid and bile salt tolerance, bile salt hydrolase activity and cholesterol-lowering properties) of *Lactobacillus plantarum* strains Lp10S and Lp11S isolated from "Sha'a" and to demonstrate their *in vivo* hypocholesterolaemic effects on rabbits.Furthermore, the atherosclerotic index and changes in triglycerides were also investigated.

MATERIALS AND METHODS

Bacterial strains and culture conditions: Strains Lp10S and Lp11S used in this study were obtained from the Laboratory of Biochemistry, Food science and Nutrition (LABPMAN) of the University of Dschang-Cameroon. Previously, they were isolated from "Sha'a", a Cameroonian maize-based traditionally fermented beverage and identified as Lactobacillus plantarum based on the pattern of carbohydrate fermentation and rep-PCR genomic fingerprinting results. Furthermore, they proved to be free from virulence factors like haemolytic and gelatinase activities as well as antibiotics resistance.

The strains were revived from MRS broth+glycerol (70:30 v/v) stock at-20°C and sub-cultured at least two times in MRS Broth (de Man Rogosa and Sharpe broth, Biolife, Milano, Italy) for 18 to 20 h intervals before use. The suspensions of *L. plantarum* cultures for *in vivo* trials were prepared according to the method described by Zambou *et al*. (2007); the cells were resuspended in sterile physiological saline and stored at-20°C during the experimental period.

Acid tolerance: The two strains were preliminary selected using a rapid test according to the method described by Pelinescu *et al*. (2009). For real assessment of the acid tolerance, a 24 h-old culture of each Lactobacillus strain (10^8 CFU/mL) was suspended in a citrate buffer of pH 3 (identical to human gastric pH value) for 5 h at 37°C. The suspensions were then centrifuged at 3000 rpm for 10 min at 4°C and washed twice in sterile saline solution to eliminate the citrate buffer. Cells were suspended in a physiological solution and a series of ten-fold dilutions (10^{-2} to 10^{-10}) in 0.1% peptone water was prepared. A given amount of each dilution (100 µl) was plated on to de Man Rogosa Sharpe (MRS) agar (Biolife, Milano, Italy) and incubated anaerobically in GasPak anaerobic jar (Genbox anaer; BioMérieux, France) at 37°C for 24-48 h (Verdenelli *et al*., 2009). The percentage of the viable bacteria was calculated.

Bile salt tolerance: Strains were cultured on MRS agar medium for 24 h at 37°C. Colonies were collected and suspended in 0.5 M phosphate buffer pH 7 supplemented with 0.4% (w/v) bile salts (Oxgall; Sigma, St Louis, USA) and the turbidity of suspensions adjusted to 0.5 using Mc Farland standard (1.5×10^8 CFU/mL). The resulting suspension was then centrifuged at 3000 rpm for 10 min at 4°C and washed twice in sterile saline solution. The cells were again suspended in physiological solution and serially diluted (10^{-2} to 10^{-10}) in 0.1% peptone water. From each dilution, 100 µL was plated on to de Man Rogosa Sharpe (MRS) agar (Biolife, Milano, Italy) and incubated anaerobically in GasPak anaerobic jar (Genbox anaer; BioMérieux, France) at 37°C for 48 h. Results were expressed as the percent (log CFU) of resistant cell.

Qualitative assay for deconjugation of bile salts: The strains were screened for bile salt hydrolase (BSH) activity by spotting 10 µl aliquots of overnight cultures on MRS agar plates supplemented with 0.5% (w/v) sodium salt of taurocholate (Sigma, USA) and 0.37 g/L of $CaCl_2$ (Schillinger *et al*., 2005). Plates were incubated anaerobically at 37°C for 72 h. The precipitation zone surrounding colonies indicated the bile salt hydrolase activity of bacteria. Strains were grouped into one of the three arbitrary classes based on the diameter of the precipitation zones on BSH screening medium according to Mathara *et al*. (2008): low BSH activity if the strain demonstrated precipitation zone up to 10 mm; medium BSH activity if the isolate demonstrated precipitation zone of 11 to 15 mm; high BSH activity if the isolate demonstrated precipitation zone greater than 15 mm.

Assessment of in vitro cholesterol-lowering property: The ability of strains to remove cholesterol from the culture medium was determined as described by Dora and Glenn (2002) with slight modification. A 9.9 mL aliquot of MRS broth containing 0.4% (w/v) bile salt and 0.01% (w/v) cholesterol (reference: 139 050, Boehringer, Mannheim, Gmbh) was inoculated separately with 0.1 mL overnight culture of each of the strains. The inoculated tubes were incubated at 37°C under anaerobic conditions for 18 h. The bacterial cells were removed from the culture broth by centrifugation at 4000 rpm for 20 min and the supernatant was used directly for quantifying cholesterol. Non inoculated MRS broth containing 0.4% (w/v) bile salt and 0.01% (w/v) cholesterol was used as control and each strain was tested in triplicate. Total cholesterol was measured enzymatically with a commercial specific kit (INMESCO, Germany). The amount of cholesterol removed from the growth medium was expressed as a percentage after comparison to the control as follows:

$$\%\,cholesterol\ removed = \frac{1 - residual\ cholesterol\ in\ cell\ free\ broth}{cholesterol\ of\ control\ broth}\times 100 \quad (1)$$

Cell pellet was dried at 80°C until constant weight. Cholesterol assimilation of the isolates was expressed as the amount of cholesterol consumed in milligram per gram of cells.

Table 1: Grouping of animals (n = 5) and treatments with respect to time

Groups	Week 1 to Week 7	Week 7 to week 8 (post ingestion)
To-	D	D
To+	D + Cholesterol	D
T1	D + Lp10S	D
T2	D + Cholesterol + Lp10S	D
T3	D + Lp11S	D
T4	D + Cholesterol + Lp11S	D

To-: Negative control; To+: Positive control; D: 300g Standard diet per day per rabbit; Cholesterol: 2×0.02g of pure cholesterol per day per rabbit; Lp10S: a dose of 2×10^8 CFU of *L. plantarum* Lp10S strain per day per rabbit; Lp11S: a dose of 2×10^8 CFU of *L. plantarum* Lp11S strain per day per rabbit.

Animals, diet and experimental design: This study was carried out at the rabbit unit of teaching and research farm of the Faculty of Agronomy and Agricultural science (FASA), University of Dschang-Cameroon. For this purpose, thirty male rabbits (Oryctolagus cuniculus) of New Zealand White strain aged 180±20 days, weighing 1300-1500 g were housed individually in stainless steel cages (70 cm side), in well ventilated room with constant temperature of 21±1°C, 55±5% humidity and a 12 h light/dark cycle, as per the norms of institutional animal Ethics Committee. These rabbits were initially fed ad libitum with a standard diet (which includes 23% protein, 4% fats, 49% carbohydrates, 5% fiber and 10% minerals) during an adaptation period of 14 days for acclimatization. Afterwards, random allocation was made in six experimental groups of five rabbits each (n = 5) which were treated as described in Table 1. During experimentation, cholesterol and L. plantarum cells suspensions were administered by oral gavage. The treatments lasted seven weeks followed by one week post ingestion period during which the animals were fed with the standard diet alone.

Animals were weighed weekly throughout the experimental period. According to the procedure of Diehl *et al*. (2001), blood specimens from the marginal ear vein were collected at 1-week interval from the beginning of the treatment, following food deprivation for 12 h.

Analysis of serum lipids: Blood samples collected (3 mL) were placed in haemolysis tubes with 1% of 5 M EDTA (antioxidant) and allowed to stand at room temperature until the formation of clot. After unstitching, the coagulated blood was centrifuged at 3000 g at 4°C for 10 min and the serum collected for immediate analyses.

The serum levels of Total Cholesterol (TC), HDL-Cholesterol (HDL-C), triglycerides (TG) were measured for each rabbit by enzymatic colorimetric method, with a commercial specific kit (INMESCO, Germany). Non HDL-Cholesterol (nHDL-C) was calculated by subtracting HDL-C from TC and represented the (LDL + IDL+VLDL)-Cholesterol fractions (Song *et al*., 2006; Liu *et al*., 2005).The atherosclerosis index was calculated using the following formula:

$$Atherosclerotic\ Index = \frac{(VLDL + IDL + LDL) - Cholesterol}{HDL - Cholesterol} \quad (2)$$

Statistical analysis: Results for each group were expressed as mean±standard error. One-way ANOVA was used to examine the differences between groups, thanks to SPSS 17.0 (SPSS Inc., Chicago, IL, USA) program.

RESULTS

Acid and bile salt tolerance: The tested strains demonstrated high tolerance to acidic conditions of pH 3 after 6 h of exposure in citric acid at 37°C by showing survival percentages greater than 50% (Table 2). These acidotolerant strains also showed good capacity to resist bile salts by presenting survival percentage greater than 50% under exposure to 0.4% bile salts after 6 h at 37°C (Table 2).

Deconjugation of bile salts: All the 2 strains displayed BSH activity by providing a precipitation zone around colonies on plate assay. L. plantarum Lp10S strain exhibited a higher BSH activity by expressing precipitation zone diameter greater than 15 mm (Fig. 1).

In vitro cholesterol-lowering property: The strains were tested for their ability to reduce cholesterol in vitro in the presence of bile salts. The amount of cholesterol removed ranged from 65.20 to 72.50% (Fig. 2A). Cholesterol assimilation of the isolates ranged from 26.30 to 33.18 mg of cholesterol per g of cells (Fig. 2B). *Lactobacillus plantarum* 10S showed a significantly higher cholesterol removal, potential, whereas *Lactobacillus plantarum* 11S had a greater cholesterol assimilation potential.

Total weight gain of rabbits: The rabbits were generally healthy throughout the trial period. All the

Table 2: Survival of *L. plantarum* Lp10S and Lp11S strains under acidic and high-bile salts conditions after 6h of incubation at 37°C.

	pH 3			0.4% bile salts		
Strains	Initial count(log10 UFC/mL)	Count after 6h (log10 UFC/mL)	Survival percentage (%)	Initial count (log10 UFC/mL)	Count after 6h (log10 UFC/mL)	Survival percentage (%)
Lp10S	9.67±0.25	7.97±0.12	82.42	9.67±0.25	8.40±0.17	86.86
Lp11S	9.43±0.38	6.79±0.23	72.00	9.43±0.38	7.90±0.10	83.77

Values in this table are means of three replicates.

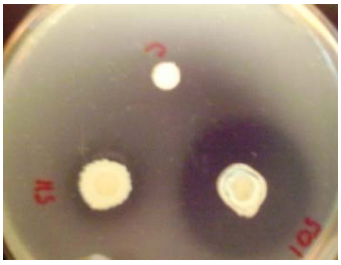

Fig. 1: BSH activity of *L. plantarum* strains Lp10S and Lp11S, grown on MRS agar supplemented with 0.5% (w/v) sodium salt of taurocholate and 0.37 g/L of $CaCl_2$ and incubated anaerobically for 48 h. **C**: control (MRS broth spotted on a 6 mm disc); **10S**: *L. plantarum* Lp10S; **11S**: *L. plantarum* Lp11S.

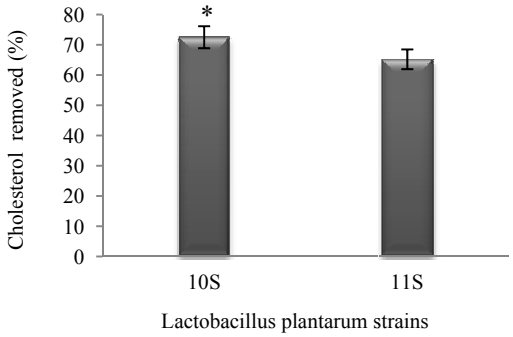

Fig. 2A: Percentage of cholesterol removed by strains of *Lactobacillus plantarum* Lp10S and Lp11S grown in MRS broth supplemented with MRS 0.4% bile salts and 0.01% of cholesterol at 37°C for 24h. *: The value with this symbol is significantly different ($p<0.05$) from the other.

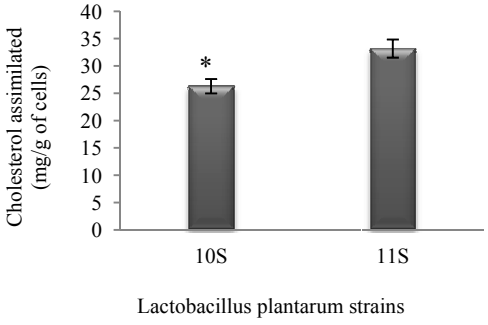

Fig. 2B: Quantity of cholesterol assimilated by strains of *Lactobacillus plantarum* Lp10S and Lp11S grown in MRS broth supplemented with MRS 0.4% bile salts and 0.01% of cholesterol at 37°C for 24 h. *****: The value with this symbol is significantly different ($p<0.05$) from the other.

groups showed no significant differences ($p>0.05$) in body weight gain. This indicates that the animals fed with L. plantarum Lp10S and L. plantarum Lp11S had growth patterns similar to the controls (Fig. 3A and B).

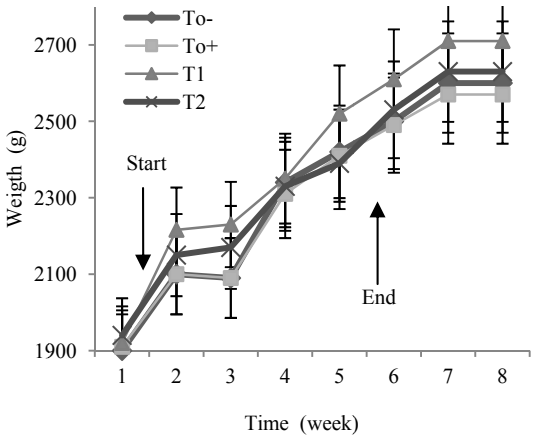

Fig. 3A: Body weight gain of rabbits fed diets containing *L. plantarum* Lp10S (T1 and T2) and those of the controls (To- and To+) during the experimental period.
Start: beginning of the treatment. End: end of the treatment

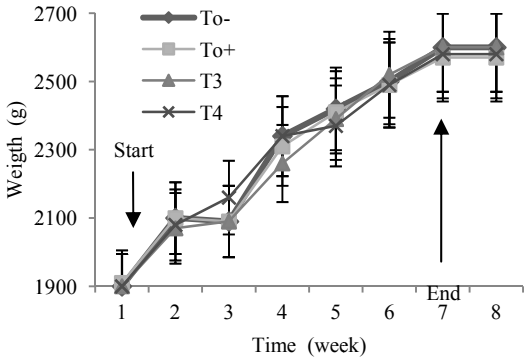

Fig. 3B: Body weight gain of rabbits fed diets containing *L. plantarum* Lp11S (T3 and T4) and those of the controls (To- and To+) during the experimental period.

Serum lipids analyses: The experiment was conducted on hypercholesterolemic rabbits, in order to clearly determine the potential of *L. plantarum* Lp10S and *L. plantarum* Lp11S to reduce the total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and atherosclerosis index.

- **Changes in total cholesterol:** The Fig. 4A and B showed that the total cholesterol levels of rabbits of groups To-, T1 and T3 were not significantly different ($p>0.05$), neither during the treatment nor after the treatment. However, rabbits of groups T2 and T4, expressed significantly lower ($p<0.05$) total cholesterol levels compared to rabbits of group To+ from the sixth week up to the end of the treatment, with a maximum reduction level of 31.5

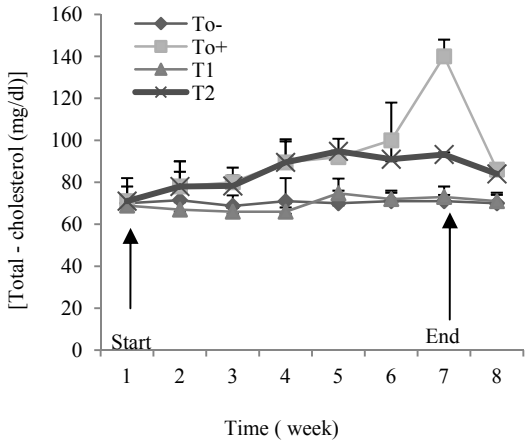

Fig. 4A: Serum total cholesterol content of rabbits fed diets containing *L. plantarum* Lp10S (T1 and T2) and those of the controls (To- and To+) during the experimental period. *: The values of To+ and T2 with the same symbol are statistically different ($p<0.05$) at weeks 5 and 6.

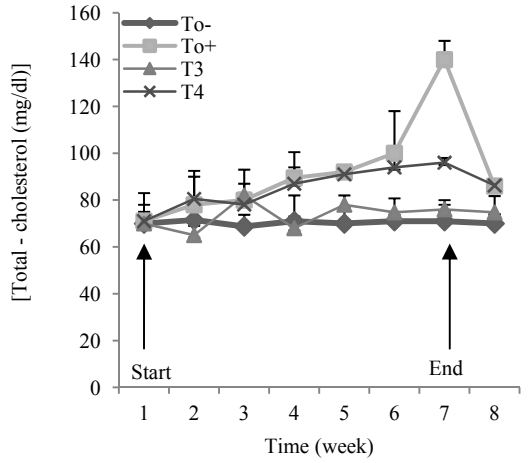

Fig. 4B: Serum total cholesterol content of rabbits fed diets containing *L. plantarum* Lp11S (T3 and T4) and those of the controls (To- and To+) during the experimental period. *: The values of To+ and T4 with the same symbol are statistically different ($p<0.05$) at weeks 5 and 6.

to 33.5 % respectively for strains Lp10S and Lp11S. After stopping the administration of cholesterol, the total cholesterol level of group To+ diminished until it attained its initial value, identical to the negative control (To^-) in 7 days.

- **Changes in HDL-cholesterol:** Changes in HDL-cholesterol of rabbits of different groups are depicted in Fig. 5A and B. The consumption of the standard diet alone (To^-) or with the two probiotics strains (T1 and T3) did not significantly modify ($p>0.05$) the level of rabbit's HDL-cholesterol.

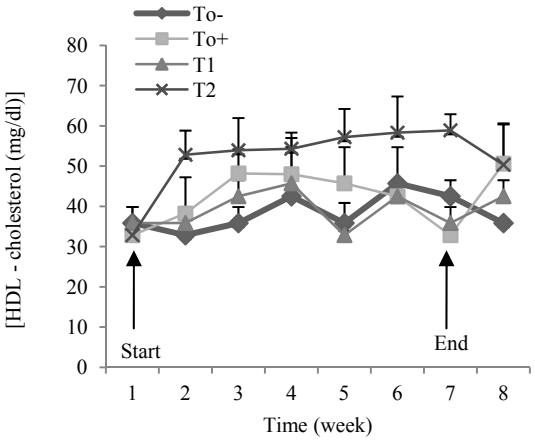

Fig. 5A: HDL-cholesterol content of rabbits fed diets containing *L. plantarum* Lp10S (T1 and T2) and those of the controls (To- and To+) during the experimental period. *: The values of To+ and T2 with the same symbol are statistically different ($p<0.05$) from weeks 2 to 7.

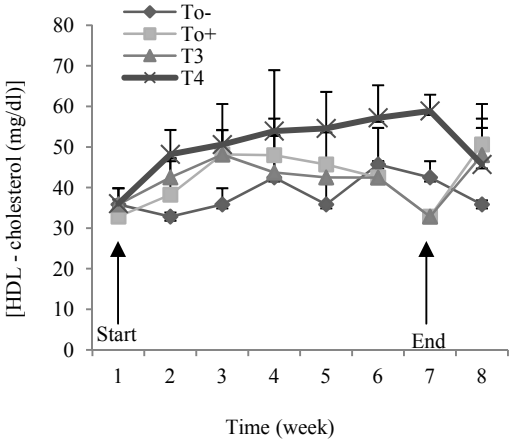

Fig. 5B: HDL-cholesterol content of rabbits fed diets containing *L. plantarum* Lp11S (T3 and T4) and those of the controls (To- and To+) during the experimental period. *: The values of To+ and T4 with the same symbol are statistically different ($p<0.05$) from weeks 2 to 7

However, for the rabbit groups whose diet included cholesterol and probiotics strains (T2 and T4) , a significant increase ($p<0.05$) of the HDL-cholesterol level (up to 79.3%) was observed, compared to the positive control (To+).

- **Changes in (VLDL+IDL+LDL)-cholesterol:** Levels of (VLDL+IDL+LDL)-cholesterol in rabbits of the six groups are shown in Fig. 6A and B. Results do not reveal any significant changes ($p>0.05$) in rabbit's (VLDL+IDL+LDL)-cholesterol levels of groups To- , T1 and T3 fed on standard diet alone or supplemented with probiotic strains during the whole experimentation. In

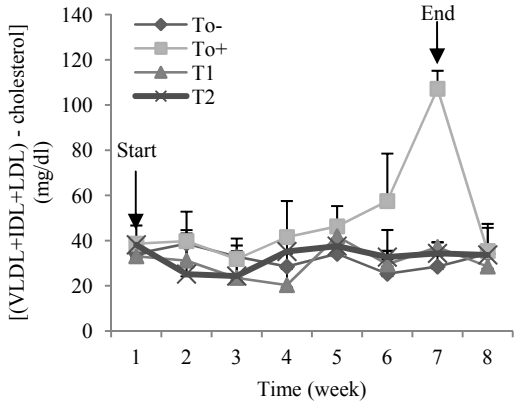

Fig. 6A: (VLDL+IDL+LDL)-cholesterol content of rabbits dosed with *L. plantarum* Lp10S (T1 and T2) and those of the controls (To- and To+) during the experimental period. *****: The values of To+ and T2 with the same symbol are statistically different ($p<0.05$) from weeks 6 to 7.

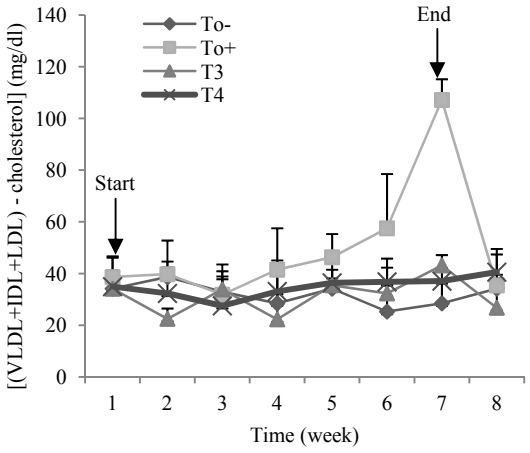

Fig. 6B: (VLDL+IDL+LDL)-cholesterol content of rabbits fed diets containing *L. plantarum* Lp11S (T3 and T4) and those of the controls (To- and To+) during the experimental period. *****: The values of To+ and T4 with the same symbol are statistically different ($p<0.05$) from weeks 6 to 7.

contrast, a significant increase in (VLDL + IDL+LDL)-cholesterol level was observed at the sixth and seventh weeks of treatment in rabbits of group To+ which received cholesterol in addition to standard died. However, for rabbits of groups T2 and T4 fed on cholesterol and probiotic strain in addition to standard diet, the (VLDL+IDL+LDL)-cholesterol levels significantly decreased ($p<0.05$) from the fifth week, compared to the positive control (To+) which received cholesterol and standard diet. The maximum reduction level was 65.4 to 69.5%.

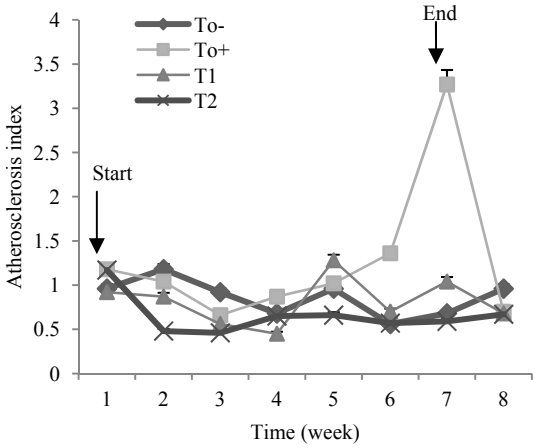

Fig. 7A: Atherosclerosis indices of rabbits fed diets containing *L. plantarum* Lp10S (T1 and T2) and those of the controls (To- and To+) during the experimental period. *****: The values of To+ with this symbol is statistically different ($p<0.05$) from all the others at weeks 6 to 7.

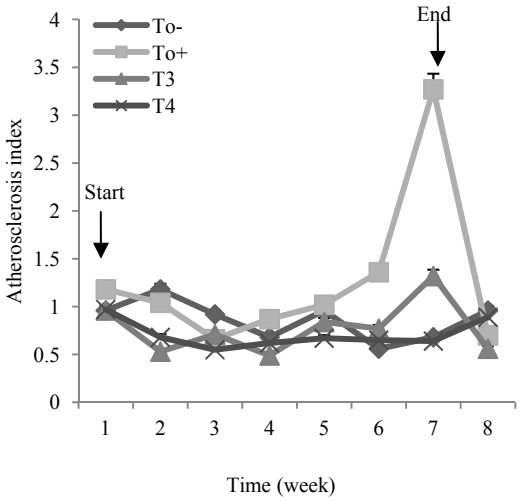

Fig. 7B: Atherosclerosis indices of rabbits fed diets containing *L. plantarum* Lp11S (T3 and T4) and those of the controls (To- and To+) during the experimental period. *****: The values of To+ with this symbol is statistically different ($p<0.05$) from all the others at weeks 6 to 7.

Atherosclerosis index: Figure 7A and B represent the effect of treatment on the atherosclerosis index. It is noticeable that this index is significantly higher ($p<0.05$) in rabbits of group To+ fed on standard diet supplemented with cholesterol in comparison to the indices of all the other groups.

- **Changes in triglycerides:** As shown in Fig. 8A and B**,** results revealed a significant increase ($p<0.05$) in the triglycerides levels of rabbits of

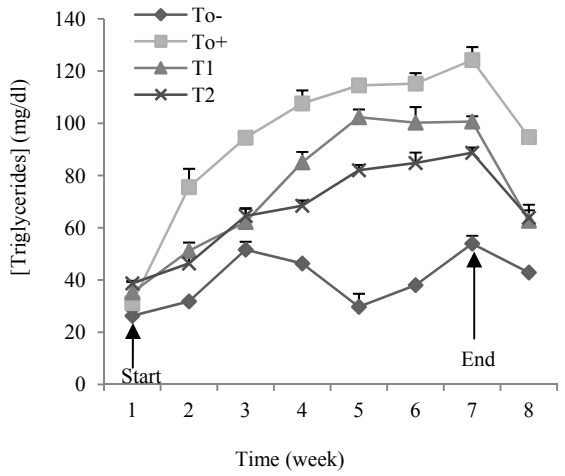

Fig. 8A: Triglycerides levels in rabbits fed diets containing *L. plantarum* Lp10S (T1 and T2) and those of the controls (To- and To+) during the experimental period. *****: The values of To+ and T2 with this symbol are statistically different ($p<0.05$) at weeks 2 to 7.

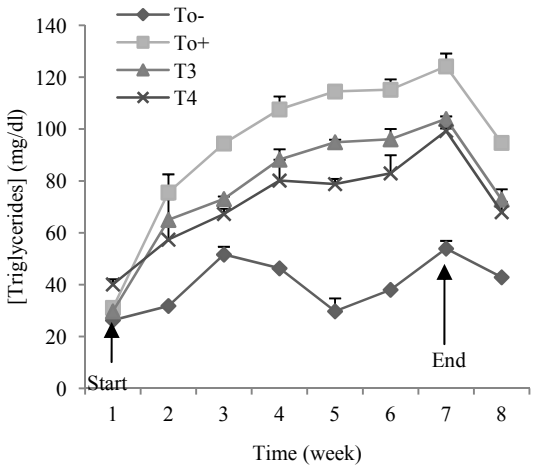

Fig. 8B: Triglycerides levels in rabbits fed diets containing *L. plantarum* Lp11S (T3 and T4) and those of the controls (To- and To+) during the experimental period. *****: The values of To+ and T4 with this symbol are statistically different ($p<0.05$) at weeks 2 to 7.

group To+ fed on standard diet supplemented with cholesterol, compared to the other groups.

DISCUSSION

Probiotics are health-promoting microorganisms. The criteria used to select potential probiotics are related to acid and bile tolerance, production of antimicrobial substances, cholesterol metabolism, production of useful enzymes and safety for food and clinical use (Ouwehand *et al*., 1999).

The ability of lactic acid bacteria to resist the effects of bile salts and acid is of great importance for their survival and growth in the stomach and the small intestine and thus, is a prerequisite for their use as probiotics (Havenaar *et al*., 1992). Strains exhibiting such properties might reach their final destination for beneficial effects. In this study, *L. plantarum* Lp10S and Lp11S strains survived under low pH conditions for 6 h and they tolerated well the bile acids under *in vitro* conditions. Many scientific reviews demonstrated the capacity of *L. plantarum* strains to tolerate gastric acidic and intestinal bile salts conditions (Kalui *et al*., 2009; Sirilun *et al*., 2010; Sieladie *et al*., 2011; Zambou *et al*., 2012) and several studies reported that tolerance to acid and other gastrointestinal stresses is strain specific (Morelli, 2000; Huang and Adams, 2004). For a strain to be considered as probiotic, it should be able to survive at pH 3 and in the presence of 0.1% bile salt (Dunne *et al*., 2001). According to this statement, the 2 strains tested in this study could be considered as potentially probiotic strains.

Bile salts are synthesized in the liver from cholesterol and are secreted from the gall bladder into the duodenum in the conjugated form in volumes ranging from 500 to 700 mL per day. The relevant physiological concentrations of human bile range from 0.1 to 0.3% (Dunne *et al*., 2001) and 0.5% (Mathara *et al*., 2008). Thus, it is necessary that efficient probiotic bacteria should be able to grow in bile salt with concentration ranging from 0.15 - 0.30% (w/v). Bile Salt Hydrolytic (BSH) activity may contribute to resistance of lactobacilli to the toxicity of conjugated bile salts in the duodenum and therefore is an important colonization factor (De Smet *et al*., 1995). The deconjugation reaction observed with *Lactobacillus plantarum* strains Lp10S and Lp11S is catalysed by conjugated bile acid hydrolase. It has been reported that this enzyme is produced exclusively by bacteria and the deconjugation ability, which reduces the toxic effects of bile salts, is widely found in many intestinal bacteria including the genera *Enterococcus, Bifidobacterium* and *Lactobacillus*. Schillinger *et al*. (2005) demonstrated that among lactobacilli isolated from probiotic yoghurts, BSH activity was only for the strains of the *L. acidophilus* group and not for *L. paracasei* and *L. rhamnosus*. Recently, Sirilun *et al*. (2010) demonstrated that among the 4 lactobacilli strains tested, BSH activity was observed in 2 strains which were identified as *L. plantarum*. However, as revealed by a study, 17 strains of *L. casei* (most probably *L. paracasei*) were found to grow in the presence of 0.5% bile without hydrolyzing the bile salts (Bertazzoni *et al*., 2004) thus indicating that bile salt hydrolase activity and resistance to bile salts are sometimes unrelated in lactobacilli.

Other hypocholesterolaemic mechanism(s) of lactobacilli may be involved in the removal of

cholesterol from growth media. The results from this study showed that *L. plantarum* Lp10S and Lp11S strains exhibited high cholesterol-binding ability, by removing 65.20 to 72.50% cholesterol from the growth medium after 18 h of incubation and demonstrated cholesterol assimilation capacity of 26.30 to 33.18 mg of cholesterol per g of cells. Other studies also reported that strains of lactobacilli tested were able to remove respectively 31.5 to 58.5% and 26.74 to 85.41% cholesterol (Hyeong-Jun *et al*., 2004; Ramasamy *et al*., 2009; Sirilun *et al*., 2010 demonstrated that the four *L. plantarum* strains isolated from food origin were considered as effective probiotics with cholesterol-lowering property capable of reducing 25.41% to 81.46% from the growth medium after 24 h of incubation.. The removal of cholesterol by lactobacilli *in vitro* could be due to an uptake or assimilation of cholesterol by bacterial strains. Liong and Shah (2005) demonstrated that a portion of the cholesterol assimilated by *Lactobacillus* strains was incorporated into the cellular membrane. Binding of cholesterol to the cells of LAB varied among the strains and species and differences in binding abilities may be due to the chemical and structural properties of their cell-wall peptidoglycans.

The strains were used to carry out *in vivo* studies in order to ascertain whether similar hypocholesterolaemic effects could be observed under *in vivo* conditions. Therefore, in order to induce hypercholesterolaemia, cholesterol was administered to rabbits in addition to the diet. High concentrations of total cholesterol and LDL-Cholesterol are highly associated with an increased risk of coronary heart disease; thus, reduction in total cholesterol and LDL-Cholesterol in hypercholesterolaemic men can reduce the incidence of cardiovascular disease (Probstfield and Rifkind, 1991). Our results showed that administration of *L. plantarum* strains Lp10S and Lp11S to rabbits with induced hypercholesterolaemia did not significantly affect the body weight. The results obtained here correlated with the findings of Bernardeau *et al*. (2002), Liong and Shah (2006) and Wang *et al*. (2009) who used *L. acidophilus, L. casei* ASCC 292 and *L. plantarum* MA2 respectively, as supplementation to a high-cholesterol diet and found a little change in body weight gain. The present study showed that administration of the 2 *L. plantarum* strains resulted in a reduction of total serum cholesterol, (VLDL+IDL+LDL)-cholesterol and triglycerides levels (31.5 to 33.5%, 65.4 to 69.5% and 24 to 38.7% respectively) of hypercholesterolaemic rabbits, bringing the levels back to standard diet values. These findings are in agreement with previous reports in rabbits (Dilmi-Bouras *et al*., 2007), in rats and in humans (Gilliland *et al*., 1985; Danielson *et al*., 1989; Usman, 2000; Bertazzoni *et al*., 2004; Suman *et al*., 2006; Wang *et al*., 2009) and *in vitro* with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Dilmi-Bouras, 2006). However, some researchers (Thompson *et al*., 1982; Grunewald and Mitchell, 1983; St-Onge *et al*., 2002) did not observe hypocholesterolaemic effect from lactic acid bacteria consumed by mice and humans. These conflicting results may be due to the experimental animals used and/or the different properties (e.g., acid, bile tolerance, different mechanisms of lowering cholesterol *in vitro*) of LAB strains used, as also stated by Akalin *et al*. (1997) and Taranto *et al*. (1998).

Conversely, HDL-cholesterol level increased by 79.30% and the atherosclerosis index was significantly lowered by 2.63 to 2.68 times. These results are in agreement with several others and confirmed the moderate action of certain lactic acid bacteria on the HDL-cholesterol (Enrica *et al*., 2000; Dilmi-Bouras and Sadoun, 2002; Klebling *et al*., 2002; Desreumaux, 2003; Dilmi-Bouras, 2006; Suman *et al*., 2006; Dilmi-Bouras *et al*., 2007). However, Tamaï *et al*. (1996), Ibrahim *et al*. (2005) and Wang *et al*. (2009) did not observe any variation in the level of blood HDL-cholesterol of rats consuming cholesterol and probiotic bacteria suspension in addition to standard diet; whereas, Chiu *et al*. (2006) reported a reduction in HDL-cholesterol in hamsters fed on high cholesterol diets. Also, Keim *et al*. (1981) and Rossouw *et al*. (1981) got similar results in humans.

Lactic acid bacteria may alter serum cholesterol by three proposed mechanisms: (a) directly binding, absorbing cholesterol into the cell and assimilation before cholesterol can be absorbed into the body (Gilliland *et al*., 1985; Noh *et al*., 1997); (b) deconjugating bile acids and produce free bile acids, which are more likely to be excreted from the body and drain the cholesterol pool as more bile acids are synthesized (Corzo and Gilliland, 1999); and (c) inhibiting HMG-CoA reductase by some metabolites of lactic acid bacteria like propionic acid (Fukushima and Nakano, 1996). The alteration of lipid metabolism in this study may be linked to primary bile salt deconjugating activities by strains Lp10S and Lp11S that could lead to reduction of the body serum cholesterol by increasing the formation of new bile acids. This hypothesis is strengthened by the fact that we found an important *in vitro* bile salt hydrolase activity with strains Lp10S and Lp 11s and reduced serum cholesterol levels in rabbits fed on the standard diet, cholesterol and *L. plantarum* Lp10S or Lp11S. Deconjugated bile salts are less efficiently reabsorbed than their conjugated counter parts, which results in the excretion of larger amounts of free bile acids in feces. Also, free bile salts are less efficient in the solubilization and absorption of lipids in the gut. Therefore, deconjugation of bile salts could lead to a reduction in serum cholesterol either by increasing the demand for cholesterol for *de novo* synthesis of bile acids to replace those lost in feces or by reducing

cholesterol solubility and thereby absorption of cholesterol through the intestinal lumen. In addition, since unconjugated bile acids are less efficient than conjugated molecules in the emulsification of dietary lipids and the formation of micelles, BSH activity may compromise normal lipid digestion and the absorption of fatty acids and monoglycerides could be impaired. The reduction of total cholesterol might also be due to cholesterol assimilation in the rabbit gut, as Klaver and Van der Meer (1993) noticed that cholesterol assimilation and bile acid deconjugase activity could occur simultaneously.

CONCLUSION

The results obtained suggest that strains of *Lactobacillus plantarum* Lp10S and Lp11S isolated from "Sha'a" a Cameroonian indigenous maize-based fermented beverage produced in western highlands of the country are potential probiotics to reduce serum levels of triglycerides, total cholesterol and (VLDL+IDL+LDL)-cholesterol therefore reducing the risk of atherosclerosis. These strains with cholesterol lowering effects could be utilized as additives for preparation of health-assistance foods. Attempts are now been made to use local food products as carriers for delivering these probiotic bacterial strains and more research is required to fully elucidate their effective mechanisms of action.

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