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Symbiotic, Hypocholesterolemic and Antioxidant Effects of Potential Probiotic Lactobacilli Strains Isolated from Tunisian Camel Milk

Imen Mahmoudi, Olfa Ben Moussa, Mnasser Hassouna

Unité de Recherche Bio-Conservation et Valorisation des Produits Agro-Alimentaires, Tunis, Tunisia
Email: imenmahmoudi15@yahoo.fr

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Abstract

In the present study, 20 selected *Lactobacillus* strains already characterized in a previous research for their capability to grow in conditions simulating the intestinal environment, their resistance to antibiotics, their antibacterial activity and their adhesion capability to intestinal human Caco-2 TC7 and HT-29 MTX cell lines, were further investigated to explore more their probiotic properties. Growth behaviour in the presence of prebiotic (fructooligosaccharides (FOS) and lactulose) at a concentration of 2%, cholesterol removal by measuring the residual cholesterol in medium supplemented with cholesterol, ability to deconjugate bile salts using BSH enzyme and antioxidant activity of culture supernatant of *Lactobacillus* strains by $ABTS^{\cdot+}$ and DPPH methods were analyzed. All probiotic strains demonstrated important prebiotic assimilation ($P > 0.05$) even with $OD_{600} > 3$ after 30 h of contact, cholesterol removal ability with maximum percentage of 57% after 24 h of contact and they were found to liberate significantly ($P < 0.05$) more cholic acid with maximum of 0.40 mM of sodium glycocholate, 0.33 mM of sodium taurocholate and 0.41 mM of their mixte and scavenge both radicals with 52% and 2.19% of $ABTS^{\cdot+}$ and DPPH respectively. This study confirmed the suitability of these probiotic strains for application in functional food formulations especially where cholesterol reduction and antioxidant activity in food are needed to assess possible in *vivo* human health benefits.

Keywords

Probiotic, Prebiotic, Cholesterol Removal, Bile Salt Deconjugation, Antioxidant Activity, Functional Food

1. Introduction

Identifying microorganisms all over the world and characterizing their metabolites are crucial steps in the study of human nutrition [1]. The term "probiotics" was defined as the active factors, when consumed in sufficient quantities, stimulating the growth of other microorganisms and apporting beneficial effects on host health [2].

Milk and dairy products have not been only characterized by their high nutritional value, but also by their ability to exert positive effects on the consumer's health as functional foods [3]. In this context, non digestible ingredients, such as prebiotics, have received important attention, because of their ability to stimulate selectively the growth and activity of probiotic bacteria in the colon [4]. However, the ability of probiotic microorganisms to use the prebiotics is strain and substrate dependent [5].

To have effects, prebiotics must escape digestion in the gastrointestinal tract and be used by the colonic microflora, mostly lactobacilli and bifidobacteria [6]; thus, after that, they are referred to bifidogenic factors [7]. Infrequently, prebiotics are reported to alleviate the virulence of pathogens like *Listeria monocytogenes* [8]. This family of compounds includes several oligosaccharides, inulin, lactulose, lactosucrose,… [9].

Bioactive components of functional foods and natural health products are responsible for their efficacy in disease prevention, therefore, they have been recommended as a favorable dietary technique to reduce cholesterol, [10]. Also, it is reported that these products reduce the risk of coronary heart disease and therefore, the characterization of the active ingredients and the type of the probiotics is very requisite [11].

Mann and Spoerry [12] were the first to demonstrate that the consumption of milk fermented with *Lactobacillus acidophilus* reduced serum cholesterol in hypercholesterolaemic African subjects. Since then, the hypocholesterolamic effect of fermented dairy products has been investigated in human nutrition [13]. These studies suggest that *Lactobacillus* strains could eliminate total cholesterol and reduce density lipoprotein (LDL) cholesterol, which is a promoter facteur on serum cholesterol levels [14].

Many researchs have revealed that ingestion of lactic acid bacteria could diminish cholesterol levels in animals [15] and humans [16]. Although the exact mechanism of cholesterol reduction by probiotic bacteria was unclear. Several mechanisms have been suggested [17], such as removal of cholesterol [18], transformation of cholesterol [17] and enzymatic deconjugation of bile salts by producing bile salt hydrolase (BSH) [19]. Besides, the hydrolyse of bile salts has often been included amongst the criteria for probiotic selection. Probiotics are able to hydrolyse glycin- or taurin-conjugated bile salts into amino acid residues and free bile salts [20]. Conjugated bile salts are usually re-circulated through enterohepatic circulation, whilst deconjugated bile salts are less soluble and are excreted in the faeces [21]. The excreted bile salts are replaced by new bile salts formed from cholesterol in the blood stream. Thus, the more bile salt excreted,

the more cholesterol is removed from the blood stream [22]. Many studies have shown that some strains of *Lactobacillus acidophilus* secrete BSH and deconjugate bile salts [22]; however, others have found no relationship between the amount of *in vitro* cholesterol removal and the degree of bile salt deconjugation [23]. These conflicting results lead to the suggestion of another mechanism, which may be associated with cholesterol assimilation from the media into the cellular membrane of probiotic bacteria during growth [17].

Also, antioxidant activity is one of the essential roles of probiotics which consists in the protection of cells and tissues from oxidation damage [24]. Thus, the aim of this study was to evaluate the growth behaviour in the presence of prebiotic carbohydrates, cholesterol removal ability via cholesterol assimilation, bile salt deconjugation and antioxidant properties of twenty *Lactobacillus* strains.

2. Materials and Methods

2.1. Bacteria and Growth Conditions

Twenty probiotic strains isolated from camel milk were identified and characterized in previous research of Mahmoudi *et al.* [25] with modified nominations. These included fourteen *Lactobacillus fermentum* strains CAT2 (B2), CAT19 (B19), CAT23 (B23), CAT28 (B28), CAT29 (B79), CAT40 (B90), CAT43 (B93), CAT47 (B97), CAT53 (B103), CABA4 (B104), CABA7 (B107), CABA16 (B116), CABA42 (B142) and CABA56 (B156) and six *Lactobacillus plantarum* CAKA28 (B128), CAKA34 (B134), CAN23 (B143), CAN29 (B149), CAN36 (B166) and CAN38 (B174). The stock cultures were stored in 20% of glycerol at −80˚C. The cultures were activated by triplicate subculturing in de Mann Rogosa Sharpe (MRS) broth (Biokar Diagnostics) using 1% of inoculum and incubation at 37˚C for 18 - 24 h.

2.2. Growth in the Presence of Prebiotics

Two prebiotics carbohydrates were used in this study; Fructoolisaccharides and lactulose (Sigma, France). Glucose was used as control growth carbohydrate. The analysis of growth capability in the presence of this carbohydrates of *Lactobacillus* strains was determined according to Pennachia *et al.* [26] with modificationss. 1 ml of each overnight culture was transferred into a sterile tube and centrifuged (1000 rpm, 10 min, 4˚C). The pellet was then resuspended in 50 ml of MRS broth and containing 2% (w/w) of each prebiotic or control carbohydrate (glucose). Growth of probiotic strainsin presence of glucose and in presence of prebiotics were monitored after 0, 8, 24 and 30 h of incubation at 37˚C by measuring the absorbance at 600 nm.

2.3. Screening of Probiotic *Lactobacillus* Strains for Cholesterol Removal

Cholesterol removal by probiotic *Lactobacillus* strains was determined according

to the spectrophotometer method which previously described by Miremadi *et al.* [14]. 30 mg of water-soluble cholesterol (poly-oxyethanyl-cholesterol sebacate) (Sigma, France) were dissolved in 10 ml of milli-q water and filter-sterilized to obtain a stock solution of cholesterol. The MRS broth containing 0.3% of bile salt (oxgall; Sigma, France) and 100 µl/ml of cholesterol solution was inoculated with 1% of probiotic cultures and incubated at 37˚C for 24 h. After 6, 12, and 24 h interval incubation, each culture was centrifuged at 4000 rpm for 20 min at 4˚C. After that, the mixture of 1 ml of the supernatant, 1 ml of KOH (33%, w/v) and 2 mL 96% ethanol was vortexed for 1 min followed by incubation at 37˚C for 15 min, and cooled to room temperature. Then, 2 ml of milli-q water and 3 ml of hexane were added to the mixture. For separation of the two layers, the upper hexane layer was collected and evaporated under nitrogen gas. Two millilitres of *o*-phthalaldehyde solution (Sigma, France) were added. To this, 0.5 ml of sulphuric acid (98%; Sigma, France) was added and vortexed for 1 min followed by resting for 10 min at room temperature before measuring the absorbance at 550 nm. The ability of probiotics to assimilate cholesterol was expressed as the percentage of cholesterol removed at each incubation interval as follows:

$$\begin{aligned}&\%\ \text{of cholesterol removed}\\&=\frac{100-\text{residual cholesterol at each incubation interval}}{100}\times 100\end{aligned}$$

The cholesterol concentration was determined using a standard curve.

2.4. Deconjugation of Bile Salts: Ammount of Free Cholic Acid

The concentration of cholic acid was determined using spectrophotometer method as previously described by Miremadi *et al.* [14]. 10 ml of MRS broth were supplemented with 6 mM of sodium glycocholate, 6 mM of sodium tauro-cholate, and a mixture of 2.8 mM of sodium glycocholate and 1.2 mM of sodium taurocholate [27]. Each mixture was inoculated with 1% activated probiotic followed by incubation for 24 h at 37˚C. After incubation, media was adjusted to pH 7.0 with NaOH (1 M) and centrifugated at 4000 rpm for 20 min at 4˚C. The supernatant was adjusted to pH 1.0 with HCl (5 M). 1 ml from each supernatant was mixed with 2 ml of ethyl acetate (Sigma, France), vortexed and the ethyl acetate layer was transferred into glass tubes and evaporated at 60˚C. 1 ml of NaOH (0.01 M) was added to dissolve the residue, then, 1 ml of furfuraldehyde (1% ; v/v) (Sigma, France) and 1 ml of sulphuric acid (8 M). Glacial acetic acid was added upon cooling to the mixture after heating at 65˚C for 10 min. The amount of cholic acid was determined at 660 nm using exter-nal cholic acid standard curve.

2.5. Antioxidant Capacity Using ABTS$^+$ Method

The antioxidant activity was determined using ABTS$^+$ (2,2 azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid)) radical cation method which previously des-

cribed by Rossini *et al.* [28] and Pieniz *et al.* [24]. $ABTS^+$ was produced by reacting ABTS stock solution with potassium persulfate (2.45 mM) (final concentration) and allowing the mixture to stand at room temperature for 16 h before use. This radical was dissolved in water (7 mM). After that, the solution was used for a maximum of three days. Before anlyse, $ABTS^+$ solution was diluted with ethanol, to an absorbance of 0.700 - 0.020 at 734 nm.. Ascorbic acid was used as the standard in the range between 0 - 9 mg/ml. After addition of 10 ml of each sample (or standards) in 1 ml of $ABTS^+$ solution, the absorbance was determined for 5 min. In another hand, ultrapure water was used as control. The percentage (%) inhibition of absorbance at 734 nm was deterined using ascorbic acid standard curve.

2.6. Scavenging Ability on 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radicals

This method was based on the capture of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) by antioxidants, producing a decrease in absorbance at 515 nm [29]. The DPPH was dissolved at a concentration of 60 mM in methyl alcohol. After homogenized, the solution was, transferred to a dark glass bottle and used only in the day of test. Then, 0.1 ml of each sample (culture supernatant) was transferred to test tube containing 3.9 ml of DPPH radical solution and homogenized by agitation. In another hand, a solution prepared from methyl alcohol (50%), acetone andwater (70%), mixed with 3.9 ml of DPPH solution was used as control. Methyl alcohol was used as a blank. The results were determined using standard curve and expressed as EC50 (μg/ml), which is the minimum concentration to reduce 50% of the initial DPPH reaction from the time the extract reached stability.

2.7. Statistical Analysis

One-way ANOVA was employed to investigate these experiences followed by multiple mean comparisons Student's test. Results were presented as the mean ± standard deviation of three repetitions. A P value < 0.05 was considered statistically significant using SPSS 20.0.

3. Results

3.1. Fermentation Capability of Prebiotic Carbohydrates

The growth capability in medium containing 2% of prebiotic carbohydrates (FOS, and lactulose) and control (glucose) was tested for the 20 probiotic *Lactobacillus* strains by measuring absorbance at 600 nm at four times of incubation at 37˚C. After 30 h the data demonstrated no significative differences ($P > 0.05$) in the bacterial growth rate (**Table 1**).

In the presence of glucose, all the tested strains showed a very good growth behaviour during the 30 h of incubation at 37˚C, with OD600 values > 2.6. The strains CAT19, CABA16 and CABA42 were to reach OD600 value > 3.0 in this media. Also, They required after 8 h of incubation the stationary growth phase.

Table 1. Growth of probiotic *Lactobacillus* strains in the presence of 2% of glucose. 2% of fructoolgosaccharides and 2% of lactulose.

Probiotic strains	Absorbance (600 nm)											
	Glucose				Fructooligosaccharides				Lactulose			
	0 h	8 h	24 h	30 h	0 h	8 h	24 h	30 h	0 h	8 h	24 h	30 h
L. fermentum												
CAT2	0.09 ± 0.01^a	2.46 ± 0.014^a	2.74 ± 0.1^a	2.74 ± 0.1^a	0.03 ± 0.1^a	1.9 ± 0.1^a	1.65 ± 0.1^a	1.69 ± 0.1^a	0.08 ± 0.1^a	2.4 ± 0.07^a	2.7 ± 0.1^a	2.71 ± 0.01^a
CAT19	0.24 ± 0.07^a	2.95 ± 0.1^a	3.04 ± 0.1^a	3.07 ± 0.02^a	0.17 ± 0.1^a	1.85 ± 0.1^a	2.88 ± 0.1^a	2.84 ± 0.1^a	0.2 ± 0.1^a	2.9 ± 0.1^a	3 ± 0.1^a	3 ± 0.01^a
CAT23	0.17 ± 0.1^a	2.86 ± 0.1^a	2.91 ± 0.07^a	2.92 ± 0.1^a	0.1 ± 0.1^a	1.56 ± 0.1^a	1.6 ± 0.1^a	1.62 ± 0.02^a	0.16 ± 0.08^a	2.81 ± 0.1^a	2.87 ± 0.1^a	2.88 ± 0.01^a
CAT28	0.2 ± 0.1^a	2.88 ± 0.13^a	2.94 ± 0.1^a	2.97 ± 0.13^a	0.15 ± 0.1^a	1.75 ± 0.1^a	1.81 ± 0.1^a	1.87 ± 0.02^a	0.18 ± 0.01^a	2.84 ± 0.1^a	2.9 ± 0.016^a	2.93 ± 0.01^a
CAT29	0.11 ± 0.11^a	2.55 ± 0.01^a	2.66 ± 0.1^a	2.66 ± 0.1^a	0.07 ± 0.1^a	1.48 ± 0.01^a	1.52 ± 0.1^a	1.58 ± 0.02^a	0.1 ± 0.1^a	2.5 ± 0.1^a	2.6 ± 0.1^a	2.63 ± 0.01^a
CAT40	0.14 ± 0.12^a	2.87 ± 0.17^a	2.92 ± 0.1^a	2.97 ± 0.09^a	0.1 ± 0.02^a	1.77 ± 0.01^a	1.82 ± 0.1^a	1.91 ± 0.1^a	0.09 ± 0.1^a	2.8 ± 0.1^a	2.87 ± 0.1^a	2.89 ± 0.01^a
CAT43	0.16 ± 0.05^a	2.86 ± 0.1^a	2.88 ± 0.11^a	2.88 ± 0.01^a	0.08 ± 0.1^a	1.79 ± 0.01^a	1.8 ± 0.1^a	1.82 ± 0.13^a	0.12 ± 0.01^a	2.8 ± 0.1^a	2.84 ± 0.07^a	2.84 ± 0.01^a
CAT47	0.12 ± 0.01^a	2.86 ± 0.21^a	2.99 ± 0.1^a	2.99 ± 0.01^a	0.06 ± 0.1^a	1.76 ± 0.1^a	1.81 ± 0.01^a	1.85 ± 0.1^a	0.09 ± 0.01^a	2.77 ± 0.1^a	2.9 ± 0.1^a	2.92 ± 0.01^a
CAT53	0.09 ± 0.01^a	2.46 ± 0.02^a	2.84 ± 0.15^a	2.87 ± 0.01^a	0.04 ± 0.1^a	1.33 ± 0.1^a	1.68 ± 0.01^a	1.69 ± 0.1^a	0.07 ± 0.01^a	2.39 ± 0.07^a	2.73 ± 0.1^a	2.8 ± 0.01^a
CABA4	0.18 ± 0.01^a	2.86 ± 0.1^a	2.9 ± 0.01^a	2.95 ± 0.1^a	0.08 ± 0.13^a	1.72 ± 0.1^a	1.81 ± 0.01^a	1.87 ± 0.1^a	0.15 ± 0.01^a	2.84 ± 0.1^a	2.85 ± 0.1^a	2.85 ± 0.01^a
CABA7	0.16 ± 0.01^a	2.96 ± 0.1^a	2.97 ± 0.21^a	2.97 ± 0.05^a	0.1 ± 0.1^a	1.9 ± 0.1^a	1.84 ± 0.1^a	1.91 ± 0.1^a	0.11 ± 0.1^a	2.92 ± 0.02^a	2.92 ± 0.1^a	2.93 ± 0.1^a
CABA16	0.28 ± 0.01^a	3.06 ± 0.02^a	3.22 ± 0.1^a	3.27 ± 0.1^a	0.25 ± 0.02^a	1.86 ± 0.1^a	2.91 ± 0.1^a	3 ± 0.1^a	0.26 ± 0.06^a	3 ± 0.1^a	3 ± 0.1^a	3.11 ± 0.1^a
CABA42	0.18 ± 0.01^a	2.89 ± 0.1^a	3 ± 0.1^a	3.01 ± 0.07^a	0.1 ± 0.01^a	1.7 ± 0.1^a	1.9 ± 0.1^a	2.31 ± 0.1^a	0.11 ± 0.1^a	2.8 ± 0.1^a	2.84 ± 0.1^a	2.85 ± 0.07^a
CABA56	0.12 ± 0.01^a	2.86 ± 0.1^a	2.94 ± 0.1^a	2.97 ± 0.1^a	0.02 ± 0.01^a	1.4 ± 0.1^a	1.5 ± 0.1^a	1.67 ± 0.1^a	0.1 ± 0.1^a	2.82 ± 0.1^a	2.84 ± 0.1^a	2.92 ± 0.1^a
L. plantarum												
CAKA28	0.1 ± 0.1^a	2.84 ± 0.1^a	2.9 ± 0.07^a	2.9 ± 0.1^a	0.03 ± 0.1^a	1.64 ± 0.1^a	1.7 ± 0.1^a	1.74 ± 0.1^a	0.08 ± 0.1^a	2.79 ± 0.1^a	2.85 ± 0.1^a	2.85 ± 0.1^a
CAKA34	0.11 ± 0.1^a	2.84 ± 0.15^a	2.88 ± 0.13^a	2.95 ± 0.02^a	0.05 ± 0.01^a	1.72 ± 0.1^a	1.72 ± 0.1^a	1.8 ± 0.1^a	0.1 ± 0.1^a	2.78 ± 0.1^a	2.78 ± 0.1^a	2.83 ± 0.1^a
CAN23	0.1 ± 0.1^a	2.8 ± 0.02^a	2.8 ± 0.1^a	2.87 ± 0.1^a	0.01 ± 0.01^a	1.27 ± 0.1^a	1.28 ± 0.1^a	1.57 ± 0.1^a	0.017 ± 0.1^a	2.78 ± 0.1^a	2.78 ± 0.1^a	2.8 ± 0.1^a
CAN29	0.1 ± 0.1^a	2.8 ± 0.1^a	2.84 ± 0.1^a	2.91 ± 0.1^a	0.011 ± 0.07^a	1.3 ± 0.1^a	1.41 ± 0.1^a	1.61 ± 0.1^a	0.04 ± 0.1^a	2.6 ± 0.07^a	2.74 ± 0.1^a	2.88 ± 0.1^a
CAN36	0.13 ± 0.1^a	2.9 ± 0.1^a	2.95 ± 0.1^a	2.97 ± 0.1^a	0.1 ± 0.02^a	1.65 ± 0.13^a	1.7 ± 0.1^a	1.77 ± 0.1^a	0.09 ± 0.1^a	2.84 ± 0.1^a	2.85 ± 0.1^a	2.87 ± 0.02^a
CAN38	0.17 ± 0.1^a	2.94 ± 0.1^a	2.94 ± 0.1^a	2.97 ± 0.1^a	0.1 ± 0.14^a	1.77 ± 0.1^a	1.8 ± 0.1^a	1.86 ± 0.1^a	0.15 ± 0.1^a	2.91 ± 0.1^a	2.92 ± 0.1^a	2.96 ± 0.1^a

Means are similar ($P < 0.05$), they are indicated by the same letter "a".

The 20 strains were all able to ferment the both prebiotics FOS and lactulose. The small increase in the final OD600 values obtained after 30 h of incubation in the presence of this prebiotic was presumably due to the insufficient purity of the carbohydrates. The growth capability in the presence of FOS was particularly good for both *Lactobacillus* strains CAT19 and CABA16, with OD600 values of 2.84 ± 0.1 and 3 ± 0.1 respectively after 30 h of incubation. Also the lactulose allowed a good fermentation by the all strains, for obtaining an OD600 value even 3.0. FOS and lactulose were an effective growth substrates for all the 20 *Lactobacillus* strains, that reached OD600 values > 3.0. Compared to the FOS, lactulose had particularly more fermented by the *Lactobacillus* bacteria.

3.2. Cholesterol Removal by Probiotic *Lactobacillus* Strains

The reduction of cholesterol by 20 probiotic strains grown in medium supple-

mented with 0.3% oxgall is shown in Figure 1. All these were able to assimilate cholesterol at varying percentages. Analysis of variance showed that the percentage of cholesterol assimilation varied significantly ($P < 0.05$) amongst probiotic strains even at the same incubation times (12 and 24 h). The cholesterol assimilation ranged 15% - 20%, 28% - 40% and 34% - 58% after 6 h, 12 h, and 24 h of incubation, respectively. In particular, both probiotic *Lactobacillus fermentum* strains CAT19 and CABA16 showed significantly ($P < 0.05$) higher cholesterol removal ability (49% ± 0.01% and 58% ± 0.01%, respectively) at 24 h of incubation as compared with the other strains. For all probiotic strains, the percentage of cholesterol removal increased significantly ($P < 0.05$) as the incubation time increased.

3.3. Deconjugation of bile Salts: Amount of Free Cholic Acid

The deconjugation activities of 20 probiotic *Lactobacillus* strains towards sodium glycocholate, sodium taurocholate and their mixture are shown in Figure 2. All probiotics were able to deconjugate both salts and their mixture with varying amounts. The amount of liberated cholic acid varied significantly ($P < 0.05$) and ranged from 0.12 to 0.40 mM for sodium glycocholate, 0.1 - 0.33 mM for sodium taurocholate and 0.15 - 0.41 mM for their mixture. Particularly, the *L. fermentum* strains CAT19 and CABA16 showed significantly higher bile salt deconjugation activities compared with other strains ($P < 0.05$). All probiotics deconjugated sodium glycocholate to a higher amounts than taurocholate and their mixture ($P < 0.05$). Student's test indicated significant differences ($P < 0.05$) between probiotic bacteria in deconjugation of the same bile salt.

3.4. Antioxidant Activity

The antioxidant activity was evaluated by two different methods: $ABTS^{\cdot +}$ and DPPH and. The culture supernatant of 20 probiotic *Lactobacillus* strains exhibited high abilities to scavenge the radical $ABTS^{+}$ ($P < 0.05$). These activities

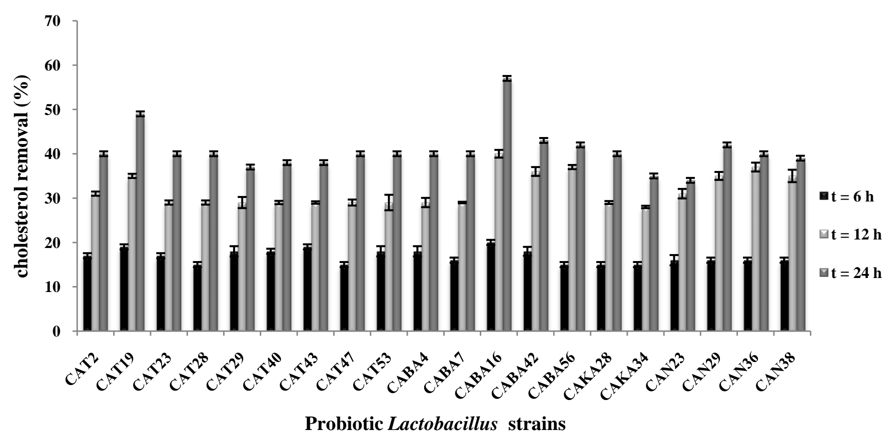

Figure 1. The cholesterol removal of 20 probiotic *Lactobacillus* strains inoculated in MRS supplemented with 100 μg/mL water-soluble cholesterol and 0.3% oxgall for 6, 12 and 24 h at 37˚C.

ranged from 19% ± 1.4% to 52% ± 1.57% (Figure 3). For DPPH method, the analysis showed significative variable percentage ($P < 0.05$). We obtained a high antioxidant activities with EC_{50} ranged from important value 2.19 ± 0.1 µg/ml to 5.19 ± 0.11 µg/ml obtained for CABA16 and CAT43 respectively (Figure 4).

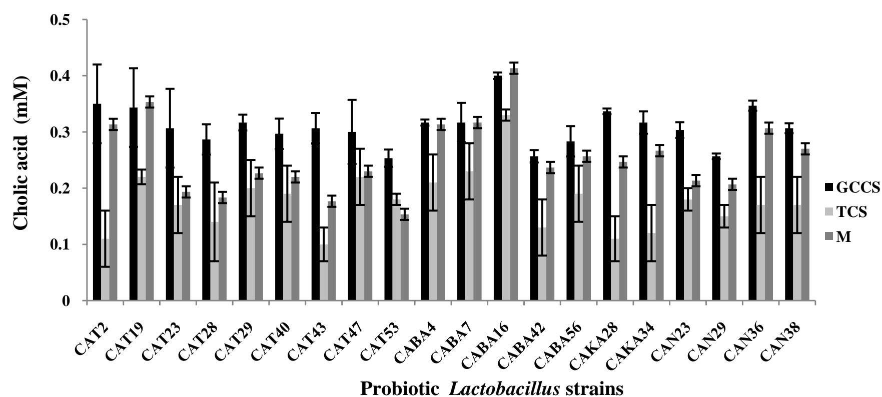

Figure 2. Bile salt deconjugation activities of the 20 probiotic *Lactobacillus* strains incubated in MRS supplemented with sodium glycocholate (6 mM), sodium taurocholate (6 mM) and their mixture (4 mM) for 24 h at 37˚C. GCCS: sodium glycocholate; TCS: sodium taurocholate; M: Mixture.

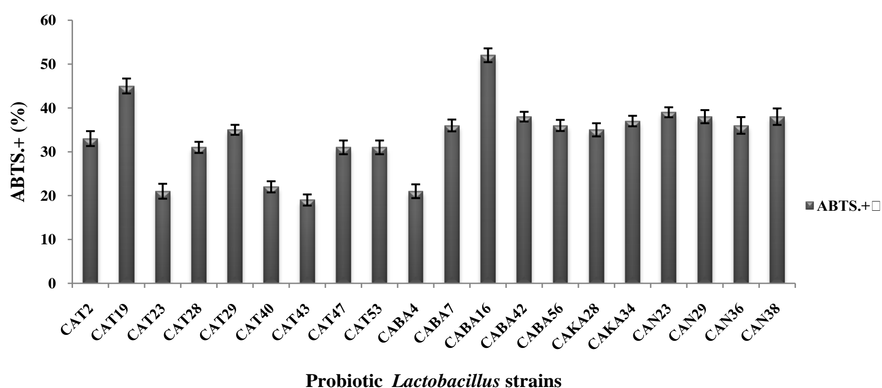

Figure 3. Antioxydant activities of 20 probiotic strains by $ABTS^+$ method.

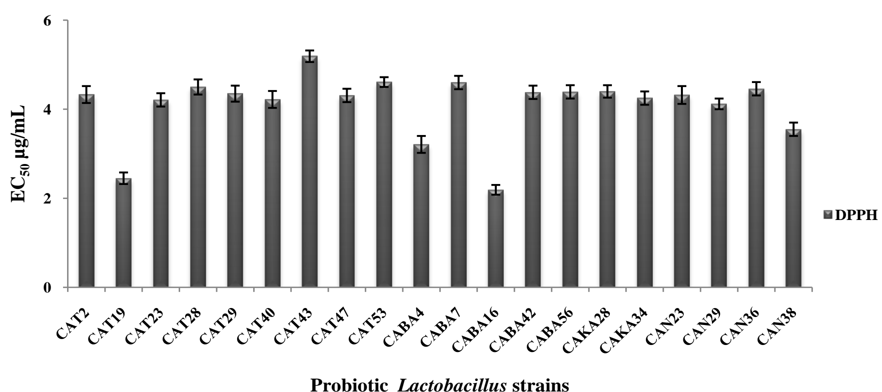

Figure 4. Antioxydant activities of 20 probiotic *Lactobacillus* strains by DPPH method EC_{50}: Minimum antioxidant concentration required to reduce the initial DPPH reaction by 50%.

4. Discussion

Many researchs have interested in the positive effects of consumption of oligosaccharides on human health. Then, the aim of supplementation of prebiotic carbohydrates is the beneficial management of the gut microbiota [30]. It have demonstrated, in *vivo*, a significant increase of the colonic bifidobacteria due to the consumption of prebiotic [31]. Oligosaccharides are present in many products, such as fruits and milk and their adjunction in functional foods has been more increased [32]. So, by producing fermented foods with the suitable prebiotic, it is possible to improve the best growth of probiotic strains added as starter cultures.

In the present study, the fermentation of prebiotics by 20 *Lactobacillus* strains was analysed. In fact, all these strains were able to ferment both FOS and lactulose. Our results were similar to those obtained by Pennachia *et al.* [26] who cofirmed that *L. plantarum* and *L. paracasei* were able to assimilate FOS and lactulose with value of $OD_{600} \geq 3$. Moreover, Saarela *et al.* [33] found that lactulose was the best fermented hydrocarbure by *L. rhamnosus* strains. In our study, the fermentation capability of prebiotics was not strain-dependent. In contrast, in another study on 28 lactic bacteria and bifidobacteria regarding their ability to ferment FOS was observed a species-dependent behaviour of fermentation [34]. The major studies of prebiotic effects indicated experiments done on the growth behaviour of total faecal microflora such as *Lactobacillus*, *Bifidobacterium*, *Streptococcus*,.. by batch culture.

Compared to FOS, lactulose was more assimilated by *Lactobacillus* strains. These results were similar to those revealed by Palframan *et al.* [35] who indicated a significant increase in the count of lactobacilli in fresh faecal slurry which was produced by adding lactulose to the batch culture fermenters; by contrast a significant decrease in their number when the FOS were added. The synbiotic system of prebiotic and probiotic is efficient if both components fit together [36]. Pennachia *et al.* [26] demonstrated that it would be useful to take considerably the specific utilization patterns of a probiotic before incorporating it into a product containing prebiotic substances, such as novel synbiotic fermented sausages. Also, they showed that it is possible to choose more than one prebiotic carbohydrate to stimulate the growth of a potential probiotic *Lactobacillus* strain to be included in novel starter culture in functional food.

High blood cholesterol was generally considered to be a risk for cardiovascular disease. The effects of probiotic bacteria on serum cholesterol levels have attracted much interest in recent years [37].

In our work, all tested probiotic strains were able to assimilate cholesterol. We revealed a high degree even of 57% which was obtained by *L. fermentum* strain CABA16 after 24 h of contact. These results were in accordance with other researchs which reported abilities of *Lactobacillus* and *Bifidobacteria* species even at 65% of cholesterol assimilation [10] [14]. Also, Lye *et al.* [17] demonstrated that potential probiotic bacteria *L. acidophilus* was able to diminish

blood cholesterol. Moreover, Nguyen *et al.* [38] showed a 10% of cholesterol assimilation by *L. plantarum* strains, isolated from infant faeces.

For all strains, the degree of cholesterol removal increased significantly ($P < 0.05$) as the incubation time increased. It is important to indicate that cholesterol activity would be bacterial growth and time dependent. In fact, probiotic strains growth may be influenced by cholesterol [14]. Besides, Lye *et al.* [17] reported, in their work, that the higer cholesterol assimilation was obtained in presence of oxgall than sodium salts.

There were three possible mechanisms may express the ability of probiotic strains to reduce cholesterol from the media. One was the co-precipitation of cholesterol with free bile salts [39]. A part of cholesterol was precipitated and resolubilised in the washing medium. This result was reported by Klaver and Van D er Meer [40], who indicated that cholesterol removal by lactobacilli was resulted to bile salts deconjugation by BSH activity of the probiotic strains. The second mechanism was the assimilation of cholesterol by the cells of probiotic bacteria. Cholesterol presented in fragmented-cells solution was attributed to the cholesterol assimilation by the cells of strains [41]. The third mechanism was the degradation of cholesterol by *Lactobacillus* strains. In this study, cholesterol could not be entirely recovered from the supernatant or washing fluid and fragmented-cells solution. Partial intake of cholesterol into the cells of the probiotic strain must produced, and some cholesterol may be degraded into a nutritional ingredients used for the growth of probiotic strains [14].

In this research, we enregistrated an important bile salt deconjugation for all tested probiotic strains. These results were in agreement with those obtained by Miremadi *et al.* [14] who reported that *Lactobacillus* and *Bifidobacterium* strains were able to deconjugate bile salts with a muximum of 56 mM of free cholic acids.

As surfactants, conjugated bile salts participate to cholesterol micelle formation by enhancing cholesterol absorption in the small intestine [42]. Following bile salt deconjugation, the cholesterol micelle formation would be disrupted and thus the absorption of cholesterol would be difficult [43].

We also demonstrate that all 20 probiotic cultures deconjugated sodium glycocholate more efficiently than sodium taurocholate. These results are in agreement with those obtained by Ramasamy *et al.* [44] and Miremadi *et al.* [14]. Contrary to glycine, taurine metabolism provides the production of hydrogen sulphide, which is highly toxic to the host after deconjugation [45]. Thus, the property of deconjugating glycine better than taurine is a fundamental characteristic for probiotics [46]. In our study, all strains showed higher deconjugating activity on sodium glycocholate than sodium taurocholate. It could be obvious that probiotics exercise deconjugation activities as a defensive mechanism against the toxic effects of conjugated bile salts [47]. The fact that sodium glycocholate is the predominant component of bile salts in the human intestine, it has been proposed that strains which may deconjugate sodium glycocholate may be more effective in lowering serum cholesterol [48]. With high activity, it is likely that

these strains can exert a deconjugation also in *vivo*. Furthermore, the bacterial genera which would be most likely to be used as probiotics (*Bifidobacterium* and *Lactobacillus*) are not capable of dehydroxylating conjugated bile salts, and therefore the majority of the degradation products of BSH. A probiotic strain can be precipitated and excreted in the stool [42].

Lactic acid bacteria have antioxidant mechanisms such as reduction of glutathione, NADH oxidase, NADH peroxidase, thiol compounds, metal ion chelating ability, trapping reactive oxygen species and reducing activity. These protective capacities result in antioxidant properties for certain lactobacilli bacteria and possibly provide additional dietary sources of antioxidants or probiotic bacteria capable of reducing oxidative stress [15].

In this study, the antioxidant effects of 20 probiotics were observed by two different methods, including scavenging of $ABTS^{\cdot+}$ and DPPH radicals. We showed an important activities for all strains. These results were similar to those noted by Meira *et al.* [49] and Pieniz *et al.* [24] who reported that *L. plantarum*, *L. casei* and *Enterococcus durans*, isolated from sheep, were also endowed with important antioxidant activities. It should be noted that our strains have strong potential antioxidants and can be used to reduce oxidative phenomena in food products. Nevertheless, using intact cells as delivery vehicles passing through the gastrointestinal tract, intracellular constituents released by lactic acid bacteria into the gastrointestinal tract may also be antioxidants [24].The consumption of food containing lactic acid bacteria can be recommended as healthy. It is well established that a wide variety of oxygenated free radicals are produced continuously in food and in the human body [50]. In addition to the long history of consumption, which proves the beneficial effects of probiotic lactic acid bacteria, it has been noted that these microorganisms are desirable for use in the production of various functional foods and therefore for human health.

5. Conclusion

The results of prebiotic experiments could open up to a relevant in *vivo* test that would inform if prebiotics could support the survival of a probiotic strain in the human intestine like adhesion properties. Furthermore, the current study suggests that all probiotic strains studied were able to remove cholesterol and deconjugate both sodium glycocholate and sodium taurocholate and their mixture with high degree. For another beneficial effect, these *Lactobacillus* strains exhibited antioxidant activities, which were evidenced with both methods. Therefore, investigations may be warranted to explain its potential health benefit and its application as promising probiotic strain in the feed industry especially where cholesterol reduction is the main objective.

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