African Journal of Microbiology Research Vol. 4 (10), pp. 994-1000, 18 May, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 © 2010 Academic Journals

Full Length Research Paper

Characterisation of non human origin probiotic *Lactobacillus plantarum* with cholesterol-lowering property

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Accepted 26 April, 2010

This study evaluated the probiotic and cholesterol-lowering property of *Lactobacillus* spp. isolated from non human origin. Four strains, TGCM 15, TGCM 26, TGCM 33 and TGCM 128 were selected and identified as *Lactobacillus plantarum*. These strains tolerated to 0.15% and 0.30% (w/v) bile salt and resisted to pH values 2 - 8 with survival rate more than 50% during 2 h of growth. In addition, all strains exhibited strong antimicrobial activities against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi*, *Shigella sonnei* and *Candida albicans* ATCC 90028. TGCM 15 and TGCM 33 exhibited the bile salt hydrolase (BSH) activity and cholesterol-lowering properties with the reduction rate more than 50% by active cells. In particular, TGCM 15 exhibited the significantly highest cholesterol-lowering activity at 81.46%. Conversely, TGCM 26 was determined to have the significantly lowest activity at 25.41%. The percentages of cholesterol-lowering by resting and dead cells of TGCM 15 and TGCM 33 were not significantly different. The resting and dead cells of all strains exhibited the cholesterol-lowering activities in the range of 13.11 - 23.28 and 11.44 - 19.53%, respectively. According to these results, the 4 strains of *L. plantarum* have revealed the probiotic's potential in cholesterol-lowering property.

Key words: Probiotic, *Lactobacillus plantarum*, bile salt hydrolase, cholesterol-lowering property.

INTRODUCTION

Nowadays, people are faced with a lot of health problems caused by their lifestyle. Human cardiovascular disease is the most important problem in many countries due to hypercholesterol. High cholesterol in serum and dietary are strongly associated with increased incidences of human cardiovascular diseases and colon cancer (Kim et al., 2008). Cholesterol as small as 1 mmol higher than the normal cholesterol level has been shown to increase the risk of coronary heart disease and coronary death by approximately 35 and 45%, respectively (Liong and Shah, 2005a). Reduction of total serum cholesterol of 1% can lower the risk of coronary heart disease by 2 to 3% (Pereira et al., 2003). Lactic acid bacteria (LAB) have a long and safe history of application and consumption (Holzapfel et al., 1995; Caplice and Fitzgerald, 1999). LAB such as *Lactobacillus* spp. has been associated with several probiotic effects in humans and animals (Park et al., 2007). Probiotics have been considered to have potential health-promoting benefits as biotherapeutic agents (Begley et al., 2006). One of the health-promoting benefits of probiotics is their ability to reduce blood cholesterol (Gilliland, 1985; Taranto et al., 2003; Lim et al., 2004; Liong and Shah, 2005a; Begley et al., 2006). Several studies have suggested that humans and animals origin isolated *Lactobacillus* spp. or their containing foods, influence cholesterol levels in laboratory media or living organisms (Taranto et al., 2003). Gilliland et al. (1985) have suggested that *Lactobacillus acidophilus* RP32 from fecal of pigs can grow well in the

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presence of bile and assimilate cholesterol from a laboratory growth medium. Thus, this species has the potential to inhibit increased serum cholesterol of pigs (Gilliland et al., 1985). *L. acidophilus* isolated from human intestine can remove cholesterol from laboratory media due to assimilation property. Human intestinal isolated *Lactobacillus casei* can remove cholesterol from media by means of the destabilisation and co-precipitation of cholesterol micelles (Walker and Gilliland, 1993; Brashears et al., 1998). *L. acidophilus* ATCC 43121 can incorporate and remove cholesterol from media into the cellular membrane during growth (Noh et al., 1997). A study by Pereira and Gibson (2002) revealed that human isolated *Lactobacillus fermentum* KC5b is able to remove cholesterol from the culture medium. *L. fermentum* KC5b also indicates that the hypocholesteremic effect is due to the capacity of bile salt deconjugation. From many reports, several actions of the cholesterol-lowering associated with *Lactobacillus* spp. have been described as cholesterol assimilation by the bacteria, cholesterol binding to the bacterial cell wall, and bile salt hydrolase deconjugate of bile salt (Brashears et al., 1998; Pereira and Gibson, 2002; Kim et al., 2008).

A majority of *Lactobacillus* spp., which has cholesterol-lowering ability, have been isolated from humans and animals. These species are considered to be starter culture for fermented foods, particularly milk or milk-derived products (Caplice and Fitzgerald, 1999; Mathara et al., 2008). Recently, the different kinds of plants such as vegetables, fruits, medicinal plants, grains and cereals have been produced and marketed in the form of lactic acid fermented plant beverages (FPBs) or foods for health-promoting in Thailand (Duangjitcharoen et al., 2008; Kantachote and Charernjiratrakul, 2008; Duangjitcharoen et al., 2009). However, these products are manufactured as a household-scale or as a small enterprise scale with spontaneous fermentation without starter culture.

The fermented plant beverages as cholesterol-lowering beverages have been required for health benefits. Hence, the starter inoculum probiotic with a cholesterol-lowering property will improve the quality of these FPBs. The probiotic starter from food origin, there-fore, is appropriate for use with the FPBs. The aim of this study was to isolate *Lactobacillus* spp. from food origins and to examine *in vitro* probiotic and cholesterol-lowering property. The selected strains which possess the cholesterol-lowering property will be used as starter cultures of FPBs for the future applications.

MATERIALS AND METHODS

Isolation of bacteria

Several samples of Thai fermented food products containing fish or pork were collected. A number of lactic acid bacterial strains were isolated by the dilution plate method in de Man Rogosa Sharpe (MRS) agar (Difco Detroit, USA). All isolated strains were primarily identified for *Lactobacillus* spp. and evaluated for some key probiotic properties *in vitro*. Stock cultures were maintained in 40% (v/v) glycerol at –70°C. The organisms were activated 3 times in MRS broth (Difco Detroit, USA) using 1% (v/v) inoculum at 37°C for 24 h before experimental use.

Bile salt tolerance

The bile salt tolerance test was modified from Du Toit et al. (1998) and Brink et al. (2006). MRS agar with and without bile salt were prepared. The pH of the agar was adjusted to 6.5. Overnight culture of each strain was streaked on MRS agar with 0.15 and 0.30% (w/v) bile salt (Sigma, USA). The culture plates were incubated anaerobically at 37°C for 48 h. The growth strain was mentioned as bile salt tolerant strain.

Acid tolerance

The acid tolerance of each strain was measured using a modified method of Brink et al. (2006). Overnight cultures were inoculated into MRS broth with pH previously adjusted to 2, 3, 4, 5, 8 and 9 with HCl or NaOH. At the time of 0 and 2 h incubation, each strain was cultured in modified MRS agar and was incubated anaerobically at 37°C for 48 h. Results were shown as the percentage of viable strains compared to the initial plate count at 0 h.

Antimicrobial activity to tested microorganisms

Antagonistic activity of *Lactobacillus* spp. on microbial indicators was adapted from the method of Chin et al. (2001) and Mante et al. (2003). The selected strains with bile and acid-base tolerance were cultured overnight before assay. Bacterial cultures were prepared into three fractions: normal cell supernatant, cell supernatant pH 7.0 and cell supernatant pH 7.0 containing 1 mg/ml catalase (Sigma, USA). They were investigated for antimicrobial activity by using the agar well diffusion technique. 100 µl sterilized filtrate supernatant was filled into the well against target microorganisms (provided by Department of Microbiology, Faculty of Associated Medical Sciences, Chiangmai University). After 24 h of incubation time, the diameter of the inhibition zone was measured and scored. The representation of inhibition zone were not included in 7 mm diameter of well. The inhibition zone was scored as follows: larger than 6 mm equals strong inhibition (+++), between 3 and 6 mm equals moderate inhibition (++) and less than 3 mm equals weak inhibition (+).

Bile salt hydrolase (BSH) activity

The BSH activity assay was modified from Du Toit et al. (1998) and Lim et al. (2004). Stationary phase growth of each isolate was investigated for the bile salt hydrolase activity by streaking on MRS agar plate supplemented with 0.5% (w/v) sodium salt of taurodeoxycholic acid (TDCA; Sigma, USA) and 0.37 g/l of $CaCl_2$ (Merck, Germany). Plates were incubated anaerobically at 37°C for 72 h. The precipitation zone surrounding colonies indicated the bile salt hydrolase activity of bacteria.

In vitro cholesterol-lowering property of active cell-free broth

The selected strains from previous assays were investigated for cholesterol-lowering activity. Growth culture of strains (1%) was inoculated into freshly prepared MRS supplemented with 0.3% oxgall (w/v) (Sigma, USA) as a source of bile salt. Standard

cholesterol in water-soluble form (polyoxyethanyl cholesteryl sebacate; Sigma, USA) was sterilized, filtered and added to MRS broth at a final concentration of 70 to 100 µg/ml. After 24 h of growth, the final pH of active cell culture was measured. Then, the cells were centrifuged. Spent broth was then sterilized by using 0.45 µm membrane filter (Pall Gelman Laboratory) as cell-free broth of active cells. The total amount of cholesterol in both the active cell-free broth and cell pellet were determined. An un-inoculated MRS broth at the same condition was a negative control. The cholesterol levels were determined by a small modification of the method of Rudel and Morris (1973) and Gilliland et al. (1985). The activity of cholesterol-lowering was calculated as a percentage by the treatment compared with the control (MRS broth supplemented 0.3% oxgall) as follows: [1- (residual cholesterol in cell-free broth)/(cholesterol of control broth)]×100.

In vitro cholesterol-lowering property of inactive (dead and resting) cell free broth

The method used was modified from Kimoto et al. (2002), Liong and Shah (2005a) and Marculescu et al. (2005). After 24 h of cell growth in MRS broth supplemented with 0.3% oxgall, cells were harvested by centrifuging at 10,000 × g at 4°C for 10 min. The cell pellets were washed twice with sterile distilled water and prepared into two fractions. For the resting cells preparation, the cell pellets were suspended with phosphate buffer saline (pH 7.0 ± 0.2) containing 0.3% oxgall and standard water-soluble cholesterol. For the second fraction, cell pellets were suspended with sterile distilled water and autoclaved at 121°C for 15 min. After being autoclaved, the cell pellets were suspended with MRS broth containing 0.3% oxgall and standard water-soluble cholesterol for dead cell preparation. The dead and resting cells were incubated at 37°C for 24 h. Spent broths and cell pellets of these fractions were assayed for cholesterol-lowering content as previously described.

Identification of potential isolates

The selected strains were identified according to the method of Massi et al. (2004) and Tamminen et al. (2004). *Lactobacillus* spp. strains were inoculated in MRS broth overnight at 37°C. The cell pellet was harvested by centrifuging and washed twice with PBS. The nucleic acid was extracted from the cell pellet using the UltraClean™ soil DNA isolation kit (Mo Bio Laboratories, Inc, Canada) and also purified using the high pure PCR template preparation kit (Roche, USA). PCR procedures based on 16S ribosomal DNA sequences lactobacilli genus-specific and *Lactobacillus* species-specific primers were used for identification. The PCR products were analysed by agarose gel electrophoresis.

Statistical analysis

Experiments were assayed in triplicate. Where necessary, mean ± standard deviation (SD) were presented. Statistical significance was assessed by analysis of variance (ANOVA) and then by Duncan's test with the SPSS 11.0 for Windows (SPSS Inc, Chicago, IL, USA). The level of significant difference was defined at $p < 0.05$.

RESULTS

Bile and acid tolerance

Two hundred and seventeen lactobacillus strains with gram-positive, non-spore forming and catalase-negative were isolated from 93 samples of fermented foods containing pork or fish. Among 217 lactobacilli strains, 86.64% (188/217 strains) were tolerable to 0.15% bile salt.

However, 52.53% (114/217 strains) were tolerable both to 0.15 and 0.30% bile salt. All these 114 strains were also examined for resistance to different pH values. A viable rate of more than 90% of 43 strains out of 114 strains at pH 3, 4 and 5 after 2 h of incubation was found. At the pH 2 and 8, a surviving percentage that was higher than 50% could be observed in 27 strains. All strains could survive at pH 4. In contrast, all strains could not survive at pH 9.

Antimicrobial activity against pathogenic or spoilage microorganisms

Forty-three strains with bile and acid-base tolerances were investigated for antimicrobial activity. All 3 fractions of supernatants from 18 isolates displayed antimicrobial activity against all target microorganisms such as *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi*, *Shigella sonnei* and *Candida albicans* ATCC 90028 (Table 1). Among these fractions, cells supernatant pH 7 and cell supernatant pH 7 containing catalase showed lower antimicrobial activities than normal cell supernatant fraction.

Bile salt hydrolase (BSH) activity on plate assay

Only 2 (TGCM 15 and TGCM 33) of 43 strains, which showed antimicrobial activity, displayed BSH activity by providing the precipitation zone around colonies on plate assay. These two strains were identified as *Lactobacillus plantarum*.

Change in cholesterol level of active cell-free broth

All of 4 isolated strains which were identified as *L. plantarum*, both with BSH activity and without, had ability to decrease cholesterol concentration in culture broth. 4 out of 43 strains that exhibited antimicrobial activities provided the ability to decrease cholesterol concentration in culture broth. These 4 strains were TGCM 15, TGCM 26, TGCM 33 and TGCM 128. Among these strains, TGCM 26 and TGCM 128 did not show BSH activity. Cholesterol reduction of 4 strains in active free broth ranged between 11.81 and 31.24 µg/ml. Cholesterol augmentation of active cell pellet of 4 strains ranged 2.07 - 4.77 µg/ml. Cholesterol reduction of resting cell free broth ranged 7.55 - 11.07 µg/ml. Cholesterol augmentation of resting cell pellet ranged 0.96 – 2.02 µg/ml. Cholesterol reduction of dead cell free broth ranged 6.96 - 9.77 µg/ml.

Table 1. The antimicrobial activity of lactobacilli strains against 7 microbial indicators by using agar well diffusion technique.

Strain	Fraction	Antimicrobial activity						
		E. coli ATCC 25922	*S. aureus* ATCC 25923	*B. cereus* ATCC 11778	*P. aeruginosa* ATCC 27853	*S. typhi*	*S. sonnei*	*C. albicans* ATCC 90028
TGCM 15	1	++	+++	+++	++	++	+++	+++
	2	++	++	++	++	++	++	++
	3	+	++	++	+	+	+	++
TGCM 26	1	++	++	++	++	++	++	++
	2	+	++	++	++	++	++	++
	3	+	+	+	+	+	+	+
TGCM 33	1	+++	+++	++	+++	++	++	+++
	2	++	++	++	++	++	++	+++
	3	+	++	++	+	+	++	++
TGCM 128	1	++	++	+++	+++	++	++	+++
	2	+	++	++	++	++	++	++
	3	+	+	+	+	+	+	++

Fraction 1: Cell supernatant, Fraction 2: Cell supernatant pH 7.0 and Fraction 3: Cell supernatant pH 7.0 containing catalase. +++: zone of inhibition larger than 6 mm diameter (strong); ++: zone of inhibition between 3 and 6 mm (medium); +: zone of inhibition less than 3 mm (weak); no inhibition data were not shown. Inhibition zone did not include diameter of wells (7 mm).

Table 2. Change of cholesterol content of cell-free broth and cell pellet by *Lactobacillus* spp. during a 24-h incubation of culture.

Strain	pH	Cholesterol lowering in cell-free broth and increasing in cell pellet (µg/ml)					
		Active cells		Resting cells		Dead cells	
		broth	pellet	broth	pellet	broth	pellet
TGCM 15	4.37	$31.24\pm0.52^{A,a}$	$4.77\pm0.46^{A,a}$	$11.07\pm0.22^{B,a}$	$2.02\pm0.06^{B,a}$	$9.77\pm0.35^{C,a}$	$1.81\pm0.22^{B,a}$
TGCM 26	5.46	$11.81\pm0.71^{A,d}$	$2.49\pm0.32^{A,c}$	$9.05\pm0.84^{B,b}$	$1.05\pm0.05^{B,c}$	$7.84\pm0.61^{B,b}$	$0.96\pm0.14^{B,c}$
TGCM 33	4.22	$29.70\pm0.91^{A,b}$	$3.32\pm0.40^{A,b}$	$10.15\pm0.17^{B,a}$	$1.77\pm0.13^{B,b}$	$8.98\pm0.87^{B,a}$	$1.48\pm0.10^{B,b}$
TGCM 128	4.88	$14.36\pm0.64^{A,c}$	$2.07\pm0.18^{A,c}$	$7.55\pm0.64^{B,c}$	$0.96\pm0.12^{B,c}$	$6.96\pm0.14^{B,b}$	$0.89\pm0.17^{B,c}$

^{ABC} Means of individual trials (broth or pellet) within a row with different superscript letters are significantly different ($p < 0.05$).
^{abcd} Means of individual trials (broth or pellet) within a column with different superscript letters are significantly different ($p < 0.05$).

Cholesterol augmentation of dead cell pellet ranged 0.89 - 1.81 µg/ml.

Among the tested strains, TGCM 15 had the highest significant cholesterol-lowering property in cell-free broth ($p < 0.05$). TGCM 15 also had the highest significant cholesterol-increasing activity in cell pellet ($p < 0.05$). Cholesterol in TGCM 15 pellet had the highest significant increase ($p < 0.05$) among all strains. On the contrary, TGCM 26 was the strain with the lowest significant activity ($p < 0.05$) (Table 2).

Cholesterol-lowering property of resting and dead cells

The cholesterol reduction levels from resting and dead cell free broth of all 4 strains ranged between 13.11 – 23.28 and 11.44 - 19.53%, respectively (Figure 1). The cholesterol reduction levels from resting cell free broth of *L. plantarum* TGCM 15, TGCM 26, TGCM 33 and TGCM 128 were 23.28, 17.46, 20.61 and 13.11%, respectively. The cholesterol reduction levels from dead cell free broth of *L. plantarum* TGCM 15, TGCM 26, TGCM 33 and TGCM 128 were 19.53, 13.97, 17.26 and 11.44%, respectively.

Identification of potential isolates

The four-isolated *Lactobacillus* strains isolated from different samples of fermented foods containing fish or pork were characterized and identified by the PCR procedure. The 16S DNA sequences of all 4 strains indicated that the strains are *L. plantarum*.

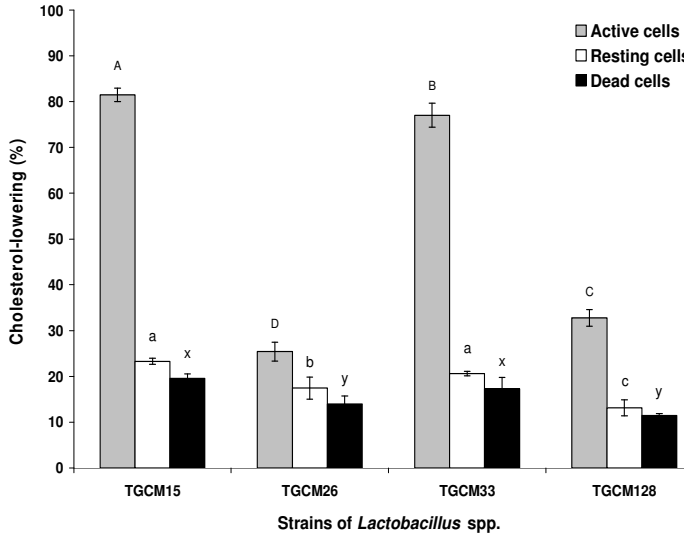

Figure 1. Percentage of cholesterol-lowering content of cell-free broth by active, resting and dead cells of *Lactobacillus* spp. after 24 h of growth. The error bars indicate the standard deviation (SD) between individual trials and different superscript letters are significantly different ($p < 0.05$) ($n = 3$).

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). Lactic acid bacteria have the most claims to be selected among potential probiotics. The pharmaceutical or functional foods are required to provide more health-promoting properties of probiotics. Therefore, this research is aimed to evaluate probiotic *Lactobacillus* strains with cholesterol-lowering property. Several strains of *Lactobacillus* spp. were isolated from fermented foods containing pork or fish. Finally, 4 strains fulfilled *in vitro* selection probiotic criteria, such as bile and acid tolerance, antimicrobial activity, activity of bile salt hydrolase enzyme (TGCM 15 and TGCM 33) and cholesterol-lowering property. *Lactobacillus* spp. strains in this study presented varying levels of survival at 0.15 and 0.30% bile concentration and pH 2 - 8. 43 strains had potential to survive under bile and acid-base environments. The strains which could tolerate bile and acidic conditions indicated the existence in stomach, intestinal juice and fermented food products. This may increase shelf life.

Resistance against pH and bile salt are important criteria in its selection for probiotic used. The organisms taken orally have to face stresses from the host which begins in the stomach, with pH between 1.5 and 3.0, and in the upper intestine and colon contain high concentrations of bile (Corzo and Gilliland, 1999). Thus, it is necessary that efficient probiotic bacteria should be able to grow in bile salt (oxgall) with concentration ranging from 0.15 - 0.30% (w/v) (Šušković et al., 2000).

As a functional probiotic, antimicrobial activity is one of the most important properties. Certain 4 strains of *Lactobacillus* spp. had ability against all 7 microbial indicators. The antagonistic activities of these strains were effective in all fractions including normal supernatant, supernatant pH 7.0 and supernatant pH 7.0 containing catalase. The antimicrobial activities of these *Lactobacillus* spp. were broad inhibitory spectrum, against yeast and bacteria both of gram-negative and gram-positive (Du Waard et al., 2002). The normal supernatant of these strains showed strong activity. While, supernatant pH 7.0 and supernatant pH 7.0 con-taining catalase showed weak antagonistic activities. The purposes of pH adjustment and catalase addition were to eliminate activity from acid and hydrogen peroxide, respectively. Although, supernatant without H_2O_2 and acid showed weak activity, it was realized that 4 strains can produce other effective metabolites except acid and hydrogen peroxide to inhibit target organisms. The activity might be due to their activities related to the amount of bacteriocin from lactic acid bacteria that are active against a number of microorganisms at the optimum pH (Du Waard et al., 2002). In general, the antimicrobial activity of lactobacilli may be due to organic acids (Kuwaki et al., 2002), hydrogen peroxide (Caplice and Fitzgerald, 1999), bacteriocins (Testa et al., 2003) or other inhibitory substances from metabolites (Caplice and Fitzgerald, 1999).

Hypercholesterolemia is considered as a major risk factor for the development of coronary heart disease (Pereira et al., 2003). Although therapeutic drugs are available to relieve this problem, they are often expensive and can have side effects. Several studies indicated that *Lactobacillus* species were able to reduce cholesterol via several mechanisms (Gilliland et al., 1985; Brashears et al., 1998; Liong and Shah, 2005a; Liong and Shah, 2005b).

The cholesterol-lowering effect of *Lactobacillus* spp. is by several means through bile salt hydrolase activity (De Smet et al., 1995; Corzo and Gilliland, 1999; Lim et al., 2004; Liong and Shah, 2005a; Begley et al., 2006). BSH is the enzyme responsible for bile salt deconjugation during enterohepatic circulation (Pereira et al., 2003; Moser and Savage, 2001). In this study, the isolated *Lactobacillus* strains were evaluated for the cholesterol-lowering activity via BSH enzyme activity and the capability of bacterial cell to remove cholesterol from culture broth. Among the 4 lactobacilli strains, TGCM 15, TGCM 26, TGCM 33 and TGCM 128, BSH activity was observed in 2 strains which were TGCM 15 and TGCM 33. Furthermore, cholesterol in broth of these 2 strains in all forms decreased, while in pellet increased. The active cells of these 2 strains exhibited significantly the highest ability ($p < 0.05$) to decrease cholesterol from broth and increase in pellet. The TGCM 15 and TGCM 33 exhibited cholesterol-lowering property higher than the other 2 strains without BSH activity. These results suggest that the

BSH ability supported the mechanism for the *in vitro* lowering of cholesterol of the cells (Parvez et al., 2006; Kim et al., 2008).

Despite that, the TGCM 26 and TGCM 128 strains did not have BSH activity but still had the ability to reduce cholesterol from cell-free broth by active cells with the percentages of 25.41 and 32.76%, respectively. This suggests that the reason for cholesterol-lowering activity of strains without BSH activity might be due to the acid produced from natural lactic acid fermentation of these lactobacilli strains. The precipitation of cholesterol in supernatant appears to be related to the deconjugation of bile salts and their subsequent precipitation at low pH which ranged from 4.22 to 5.46. The pH of the culture broth decreased due to the organic acid production by the bacteria. Bile acids are less soluble and are more likely to precipitate at pH lower than 6.0 (Klaver and Van de Van de Meer et al et al, 1993; Brashears et al., 1998). All 4 strains showed a significant change of cholesterol contents by active cells or viable cells, but not a significant difference by resting and dead cells. Cholesterol-lowering by active cells was significantly higher ($p < 0.05$) than resting and dead cells. Among 4 strains, the TGCM 15 and TGCM 33 had the higher cholesterol-lowering in cell-free broth with the percentage more than 50%. In particular, the TGCM 15 exhibited the highest significant cholesterol-lowering activity ($p < 0.05$) at 81.46%. Conversely, the strain TGCM 26 was determined to have the lowest significant cholesterol-lowering activity ($p < 0.05$) at 25.41%. These were supported by the results of experiments that the percentage of cholesterol-lowering by resting cells and dead cells (static broth condition at pH 7.0) showed lower activity than by active cells with acidic pH. This corresponded to the report that deconjugated bile salts can co-precipitate in acidic environment at pH lower than 5.5 (Klaver and Van de Meer et al, 1993; Mathara et al., 2008). This is similar to the study of Brashears et al. (1998) which reported that some lactobacilli can remove cholesterol from suspension in culture broth during growth. The greater reduction of cholesterol by active cells corresponded to the growth of cells (Liong and Shah, 2005a). Therefore, the greatest high cholesterol-lowering activities were found by active cells with BSH activity. Furthermore, the *Lactobacillus* cells were known to assimilate the cholesterol which was associated and incorporated in the cells during growth (Gilliland et al., 1985). Consistently, this study showed the increasing cholesterol in the cell pellets of all strains. Also, active cells of these BSH absent strains had the ability to associate and incorporate cholesterol in the cells during growth (Walker and Gilliland, 1993; Noh et al., 1997). The lowering cholesterol by resting and dead cells was slightly exhibited. This indicated that cholesterol might be attached via binding to cells (Liong and Shah, 2005a). In conclusion, the 4 *Lactobacillus* spp. were considered as provisional probiotic strains due to their superiority on bile salt tolerance, acid-base tolerance, antimicrobial activity, BSH activity (TGCM 15 and TGCM 33) and cholesterol-lowering property. These strains were identified as *L. plantarum. L. plantarum* have been indicated as potential probiotics with cholesterol-lowering property. This is similar to the report of Nguyen et al. (2007), who suggested that isolated *L. plantarum* from infant feces was a potential probiotic with cholesterol-lowering effects. The strain exhibits the potential to reduce mice serum cholesterol and triglyceride levels through high activity of bile salt hydrolase (Nguyen et al., 2007). From present results, the 4 *L. plantarum* isolated from food origins were considered as the effective probiotics with cholesterol-lowering property. These *L. plantarum* strains will be used as functional or bio-therapeutic agents. The authors will further study the mechanisms and activity *in vivo*. For further application, the selected strains will be used as functional starter cultures of FPBs.

ACKNOWLEDGEMENTS

The authors thank faculty of pharmacy, Chiang Mai University for providing grant and facilities. Sasithorn Sirilun gratefully acknowledges Thailand Graduate Institute of Science and Technology (TGIST), National Science and Technology Development Agency (NSTDA), Thailand.

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