Journal of Food and Nutrition Research, 2014, Vol. 2, No. 7, 340-343
Available online at http://pubs.sciepub.com/jfnr/2/7/2

DOI:10.12691/jfnr-2-7-2

Bile Salts Deconjugation Using Microencapsulated Lactic Acid Bacteria Isolated from Handmade Yogurt

Gulcin Alp Avcı*

Department Molecular Biology and Genetics/ Molecular Microbiology, Hitit University, Corum, Turkey
*Corresponding author: gulcinalp@hiti.edu.tr, alp.gulcin@yahoo.com

Received June 10, 2014; Revised June 22, 2014; Accepted June 25, 2014

Abstract The preferential aim of this study is to evaluate the deconjugation of sodium taurocholate by *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus.* As a supportive objective, determination of advantage of microencapsulation effects on deconjugation of sodium taurocholate of these strains, deconjugation property of which is the highest. Deconjugation of sodium taurocholate of *L. delbrueckii* subsp. *bulgaricus* (HL6, HL9 and HL14) and *S. thermophilus* (HS7, HS12 and HS24) strains was determined and strains with the highest deconjugation property were used for microencapsulation study. Then, viability and deconjugation of sodium taurocholate of microencapsulated strains were determined. The amount of deconjugated sodium taurocholate for *Lactobacillus* and *Streptococcus* species was found as 1.0 -1.4 mg/mL and 0.9-1.3 mg/mL, respectively. The amount of deconjugated encapsulated HL6 and HS12 strains was found 1.5 and 1.4 mg/mL, respectively. Either the viability values of encapsulated strains or the ability of deconjugation of sodium taurocholate by these strains was found much higher than those of free strains. For dairy products which were planned to be used in probiotic industry, microencapsulated strains can be recommended because of their higher viability and deconjugation ability.

Keywords: *L. delbrueckii subsp. bulgaricus, S. thermophilus, bile salt hydrolase, deconjugation, microencapsulation*

Cite This Article: Gulcin Alp Avcı, "Bile Salts Deconjugation Using Microencapsulated Lactic Acid Bacteria Isolated from Handmade Yogurt." *Journal of Food and Nutrition Research*, vol. 2, no. 7 (2014): 340-343. doi: 10.12691/jfnr-2-7-2.

1. Introduction

Lactic acid bacteria (LAB) reduce lactose intolerance, alleviation of some diarrheas, increase immune responses, prevent cancer and make blood cholesterol lowered and they have also an economic importance for food fermentation as well [1]. As lactic acid producing bacteria are being paid attention for their important roles on deconjugation of bile salts and bile salts hydrolase (BSH) activity. BSH is an enzyme which is responsible for bile salt deconjugation. This enzyme has been detected in many LAB species indigenous to the gastrointestinal tract [2]. It has also been suggested that BSH should be a requirement for the selection of probiotic organisms [3].

Many attempts have been made to elucidate the mechanism involved in the hypocholesterolemic action of LAB. One of the mechanisms is the assimilation of cholesterol by the cell wall during growth [4,5]. Another mechanism is the deconjugation of bile salts. Deconjugation is the hydrolyze of bile salts, formed by glycin and/or taurine-conjugated bile salts, into amino acid residue and free (deconjugated) bile acids by means of BSH enzyme which is produced by intestinal micro flora especially by specific groups of LAB [3,6,7]. Because of these free bile acids, such as taurocholic and glycocholic acid, these kinds of acids have weak solubility; they cannot digest by intestinal mucosa. Thus, the excretion of these free bile acids through the gastrointestinal tract will be increased as well.

Microencapsulation is a concept in which biologically active materials are encapsulated in specialized ultra-thin semipermeable polymer membranes [8]. And also, this method is a powerful technology which has been developed for use in the food industry and allows the protection of bacteria [9]. Many advantages have been demonstrated for encapsulation systems that may be applied to probiotic bacteria in the starter industries. This method is able to protect cells, making this approach potentially useful for delivery of viable bacteria to the gastrointestinal tract of humans [1]. And also, survival rate of probiotic bacteria increase during the shelf life of yoghurt and during their passage through the gastrointestinal tract with this method [11]. The encapsulation researches of probiotic bacteria may be important in dairy products containing high concentrations of viable bacteria. Increasing the viability of *L. bulgaricus* and *S. thermophilus that* used for food and health industry by encapsulation methods shall provide the ability of bile salts deconjugation for long term. Researchers have found that encapsulated and free strains of *Lactobacillus* could deconjugation tested bile salts successfully [3]. But unfortunately, no research has been found in literature about deconjugation of bile salts by encapsulated and free strains of *S. thermophilus.*

The aim of this study is primarily to evaluate the deconjugation of sodium taurocholate by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus* and secondly to determine the utility of microencapsulation effects on the deconjugation of sodium taurocholate of these strains which have the highest deconjugation property.

2. Materials and Methods

2.1. Source and Maintenance of Cultures

L. delbrueckii subsp. *bulgaricus* and *S. thermophilus* strains used in this study were obtained from the stock collection of Molecular Microbiology Laboratory at Hitit University, Faculty of Science and Arts, Department of Molecular Biology and Genetics (Corum, Turkey). They were isolated from traditionally hand-made yoghurt, identified by fermentation tests and API 50 CHL by Scientific Research Project of Hitit University (SYO01.13.001). *L. delbrueckii* subsp. *bulgaricus* strains HL6, HL9 and HL14 and *S. thermophilus* strains HS7, HS12 and HS24 were used in this study. The bacteria cultures were maintained by subculturing 1% inocula into MRS broth (De Man Rogosa and Sharpe; Merck, Darmstadt, Germany) for *L. bulgaricus* and Elliker broth for *S. thermophilus* incubating at 42°C for 18 h.

2.2. Deconjugation of Sodium Taurocholate by Strains

L. delbrueckii subsp. *bulgaricus* HL6, HL9, HL14 and *S. thermophilus* HS7, HS12, HS24 were used in this stage. MRS and Elliker broths were supplemented with 2 mg/mL sodium taurocholate (**Calbiochem**, Schwalbach, Germany). Each strain was inoculated at 1% level and incubated anaerobically at 42°C for 18-20 h. MRS and Elliker broths supplemented with 2 mg/mL sodium taurocholate alone without bacteria were used as a control assay. The amount of free cholic acid released by deconjugation of sodium taurocholate was measured for each bacteria culture. Analysis performed for free cholic acid that was released during deconjugation was carried out according to Walker and Gilliland method (1993), a modified technique of Irvin and others (1944) [12,13]. Twenty-milliliter volumes of each culture were prepared at pH 7.0 with 1N NaOH, and 5 ml distilled water was added and centrifuged at 12000xg at 1^{o}C for 10 min. 15 mL supernatant fluid was adjusted to pH=1.0 using 10N HCl and the final volume was increased to 24 mL with the addition of distilled water. Three milliliter of each samples were transferred to new glass stoppered test tubes containing 9 mL ethyl acetate (v/w). Each tube was vortexed so that phases were separated. Three milliliters of ethyl acetate layer from each tube were transferred to a clean test tube and evaporated at 60^{o}C under nitrogen gas. One milliliter of 0.01 N NaOH was added to each tube to dissolve residue. Six milliliters of 16 N H_2SO_4 were added to each tube, followed by the addition of 1 mL of 1% furfuraldehyde. Tubes were mixed, heated in water bath at 65°C for 13 min, and cooled to room temperature. Five milliliters of glacial acetic acid were added to each tube and mixed, after which the absorbance at 660 nm (BiochromLibra, UK) was read against a reagent blank. Obtained data was compared with a standard curve to determine the concentration free cholic acid (Sigma Chemical Co., St. Louis, MO, USA). Results were expressed as micromoles of cholic acid per milliliter.

2.3. Microencapsulation Procedure

Overnight strains cultures were inoculated in 500 mL of MRS and Elliker broth and incubated at 42°C for 19 h. Tubes were centrifuged at 5000 x g for 15 min and washed with PBS (pH 6.2, 1°C) for three times. Pellet was suspended with 50 mL of NaCl solution (9 g/l) and the concentrations of all bacterial suspensions were prepared according to the turbidity of McFarland standard 6 (BioMérieux – France). These suspensions mixed with a sterile Na–alginate mixture (2 mg/100 mL) and homogenized. The alginate mixed-suspensions were dropped into a sterile 0.4 mol/l $CaCl_2$ solution by a peristaltic pump. The mixture was kept at +4°C for 2 h for the separation of encapsulated sodium-alginate beads (300µm in diameter) and settled at the bottom of the bottle as calcium chloride layer [8].

2.4. Deconjugation of Sodium Taurocholate by Encapsulated and Free Strains

According to the deconjugation method, the strains performed the highest deconjugation property and coded as HL6 and HS12 were chosen and used for microencapsulation study. 80 beads were prepared from each of these two strains and put into two bottles equally (40/40). 40 beads in the first bottle were broken down in. 5 mL of 0.05 mol/l PBS (pH 6.8) supplemented with 2 mg/mL sodium taurocholate were added to each of these bottles and incubated at 42°C for 18-20 h. After incubation period unbroken beads were also broken down. For viability process, 100 µl aliquots were taken from each of both groups and viable cell counts (cfu/mL) were estimated by plating serial dilutions (10^{-1}– 10^{-8}) on MRS and Elliker agar been incubated at 42°C for 24 h. For deconjugation process, these two bottles were poured into test tubes, and then they were centrifuged at 12 000 x g for 20 min. The amount of cholic acid released from encapsulated and free bacteria were determined as described.

2.5. Statistical Analysis

All experiments were conducted in triplicate. Each value was the mean of three independent experiments. Data analysis was carried out using SPSS 15.0 bivariate correlation analysis (SPSS Inc., Chicago, IL) with statistical significance determined at $P<0.05$. The Pearson rank-order correlation test was used for comparison of the deconjugation ability of bile salts by free and encapsulated strains.

3. Results

The deconjugation of sodium taurocholate was determinate in all strains of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. Sodium taurocholate deconjugation by strains of lactobacilli and streptococci are shown by Table 1. The amount of deconjugated

taurocolic acid in the uninoculated control medium was determined as 0.68 mg/mL at the end of 19-hour-incubation time interval and the percentage of deconjugation ratios were determined according to the consideration of this loss. The amount of deconjugated sodium taurocholate was found as 1.0 mg/mL-1.4 mg/mL (ranges from 15% to 34.5%) for *Lactobacillus* species, and 0.9 mg/mL-1.3 mg/mL (ranges from 12.5% to 30.5%) for *Streptococcus* species. Among the lactobacilli strains, *L. delbrueckii* subsp. *bulgaricus* HL6 and among streptococci strains *S. thermophilus* HS12 which have the highest deconjugation values were chosen for the encapsulation process in this study. The amount of deconjugated sodium taurocholate was found as 1.5 and as 1.4 (Table 2) for encapsulated HL6 and HS12 strains, respectively. The viable cell counts of free and encapsulated strains were shown by Table 2. According to these results, either the viability values of encapsulated HL6 and HS12 strains or the ability of deconjugation of sodium taurocholate by these strains were found much higher than free strains. In addition to these results, it was observed that the viability of HL6 strain is better than that of HS12 strain.

Table 1. Amount of taurocholic acid (TCA) deconjugated and viability of all free strains

Strains	Deconjugated taurocholic acid (mg/mL)[1*]	%[2]	Viable cell counts (log10 cfu/mL)
L. delbrueckii subsp. bulgaricus HL6	1.4	34.5	9.0
L. delbrueckii subsp. bulgaricus HL9	1.3	29.5	6.2
L. delbrueckii subsp. bulgaricus HL14	0.1	15.0	3.6
S. thermophilus HS12	1.3	30.5	4.8
S. thermophilus HS7	1.2	23.5	5.2
S. thermophilus HS24	0.9	12.5	3.1

[1]The deconjugated TCA amount in the medium after the incubation period
[2]% ratio= The deconjugated TCA amount[1] - control TCA loss (0.68mg/mL)/100 x initial TCA amount (2mg/mL)
*****The mean values of triplicate measurements.

Table 2. The amount of deconjugated taurocolic acid (TCA) by encapsulated *L. delbrueckii* subsp. *bulgaricus* HL6 and *S. thermophilus* HS12 strains and viability of microencapsulated strains

Strains	Deconjugated taurocholic acid (mg/mL)[1*]	%[2]	Viable cell counts (log10 cfu/mL)[3]
HL6	1.5	42.0	12.5
HS12	1.4	35.0	8.6

[1]The deconjugated TCA amount in the medium after the incubation period
[2]% ratio: The deconjugated TCA amount[1] - control TCA loss (0.68mg/mL)/100 x initial TCA amount (2mg/mL)
[3]Log values of viable cell counts of triplicate measurements.

4. Discussion

Probiotics, when used up in efficient amounts, are living microbial supplements that improve beneficial. The beneficial effects for human health of probiotics are proven in various studies. But, in order to show the beneficial effects of probiotic bacteria to express this effect, they have adhered to the intestinal cells and colonize in here. For this reason, it has been suggested that bacteria has to be applied by encapsulating. For the encapsulation of viable bacterial cells, materials used as excipients should be gentle and non-toxic [19]. The most commonly used for microencapsulation of bacterial cells is alginate due to the fact that alginate is a cheap material and non-toxic for the body [8]. Other materials such as carrageenan, gelatin, chitosan, whey proteins, cellulose acetate phthalate, locust bean gum and starches can be used instead of alginate for encapsulation process [20]. Among the lactobacilli strains, *L. delbrueckii* subsp. *bulgaricus* HL6 and among streptococci strains *S. thermophilus* HS12 which have the highest deconjugation values were chosen to be used for the encapsulation process in this study. As a result, the number of viable cells for the encapsulated strain cultures was found higher than that of for free strain cultures. Besides, the deconjugation capacity of the microencapsulated strains has been affected positively. Therefore, encapsulation procedure should be estimated as a reliable method for the preservation of viability and permanence of probiotic functionality of probiotic strains. Similarly, it has been reported by Jones and others (2004) that microencapsulated *L. plantarum* 80 (pCBH1) strain is able to break down the conjugated bile acids, glycodeoxycholic acid and taurodeoxycholic acid effectively with BSH activity and also has the cholesterol-lowering potential [21]. Although there are many reports investigating the bile salt deconjugation ability of LAB strains isolated from human, any report couldn't be found about the same topic done by yogurt isolates of LAB [12,22].

Effects on hosts health and intestinal microbial balance. A great interest has aroused for LAB because of their probiotic properties. The *Lactobacillus* and *Streptococcus* strains are among frequently used bacterial strains in probiotic cultures [8]. Among them a combination of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, which was collaborated synergistically into yoghurt, are widely used as the major bacteria as the initiator for the production of yoghurt [14]. For this reason hand-made yoghurt isolates of *Lactobacillus* and *Streptococci* strains were chosen and used in this study.

Bile salt hydrolase (BSH), which is an enzyme, that catalyzes hydrolyses of glycine- and/or taurine-conjugated bile salts into amino acid residues and free bile acids. BSH observed widely for many gastrointestinal microbes including *Lactobacillus* spp., *B. longum*, *Bacteriodes fragilis* subsp. *fragilis* and *Clostridium perfringens* [15,16]. This enzyme acts like a part of the normal bile salt metabolism and has an importance for controlling the cholesterol levels of sera. For the assimilation of cholesterol and other lipid molecules in the intestine, deconjugated of bile salts by BSH cannot be reabsorbed from the intestine and were excreted by feces instead of being involved in blood system [17]. In recent years, a great interest has been increased for the use of BSH to influence the cholesterol metabolism of humans and farm animals. In this study, it was observed that bile salts deconjugation ability of all lactobacilli and streptococci strains could have. Although the strains used in this study were yogurt isolates, they showed BSH activity ranging from 12.5% to 34.5%. For another study performed by Pereira and others (2002), it was mentioned that release of cholic acid from sodium taurocholate depends on the production of bile salt hydrolase by bacterial strains [2]. In

the present work, we observed that elevation of the bile salt deconjugation has also increased the bile acid excretion. Bile acid concentration has been expected to be much higher in the duodenum than at the end of the ileum where the bile is secreted due to the diffusion mechanisms, microbial transformation and absorption throughout the intestinal wall [18].

According to the results, usage of microencapsulated strains as probiotic and starter can be suggested reasonably. Additionally, because of the properties mentioned before, the combined usage of HL6 and HS12 strains can be performed for high deconjugation capacity, more cholesterol lowering activity, more health benefit and stabilized fermented milk products. In a study reported by Tanaka and others (1999) which was done for determination of the BSH activity of the LAB isolated from different sources, it was stated that 59% of intestine or feces isolated and 27% of other sources isolated strains have exposed BSH activity. Depending on these results, it was suggested that the expression of BSH activity of LAB was directly correlated with the natural habitat of the bacteria [22]. Some authors suggested that deconjugation might be a detoxification mechanism for the cell, and that lactobacilli and bifidobacteria could express BSH as a protective mechanism against the toxicity of bile salts [23,24]. Free probiotic microorganisms are quite impressed with acidity of the stomach and loss of vitality is seen too much. Microencapsulation process is important providing BSH enzymes maximum viability to access to the intestinal tract. Microencapsulation enables high level protection of bacteria from stomach acidity [8,10]. Capsules, as they proceed through the gastrointestinal tract, begin to be deformed, but bacteria, due to encapsulation, are almost never affected in terms of viability [8,20]. When capsules, due to acid and bile, are completely destroyed at the time of access to the intestine, surviving bacteria resume to live and to exhibit probiotics features [20]. Thus bacterial BSH enzyme may show activity.

In conclusion, it was determined that all of the strains had deconjugation ability even if their capacities differ from each other. It was observed that encapsulated forms could keep on their viability better than the free strains and also their deconjugation ability was not decreased. As a conclusion, for dairy products which are planned to be used in probiotic industry, the encapsulated forms of *L. delbrueckii* subsp. *bulgaricus* HL6 and *S. thermophilus* HS12 strains can be recommended as appropriate because of their higher viability and deconjugation ability.

Acknowledgement

This study supported as financial by Science Research Department of Hitit University, 2013 (SYO01.13.001).

References

[1] Soomro A.H, Masud J, Anwaar K, "Role of Lactic Acid Bacteria (LAB) in Food Preservation and Human Health", *Pakistan Nutrition*, 1, 20-24, 2002.

[2] Pereira D.I.A, Gibson G.R, "Effects of Consumption of Probiotics and Prebiotics on Serum Lipid Levels in Humans", *Crit Rev Biochem Mol Biol* 37(4), 259-281, 2002.

[3] Nguyen T.D.T, Kang J.H, Lee M.S, "Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects", *Int J Food Microbiol*, 113, 358-361, 2007.

[4] Noh D.O, Kim S.H, Gilliland S.E, "Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121". *J Dairy Sci*, 80, 3107-3113, 1997.

[5] Kumar R, Grover S, Batish V.K, "Hypocholesterolaemic effect of dietary inclusion of two putative probiotic bile salt hydrolaseproducing *Lactobacillus plantarum* strains in prague-Dawley rats", *Br J Nutr*, 105, 1-12, 2010.

[6] Kim G.B, Yi S.H, Lee B.H, "Purification and characterization of three different types of bile salt hydrolase from *Bifidobacterium* strains", *J Dairy Sci*, 87, 258-266, 2004.

[7] Noriega L, Cuevas I, Margolles A, de los Reyes-Gavila´n C.G, "Deconjugation and bile salts hydrolase activity by *Bifidobacterium* strains with acquired resistance to bile". *Int Dairy*, 16: 850-855, 2006.

[8] Aslim B, Alp G, "The effect of immobilization on some probiotic properties of *Streptococcus thermophilus* strains", *Ann Microbiol*, 59 (1): 127-132, 2009.

[9] Borgogna M, Bellich B, Zorzin L, Lapasin R, Cesaro A, "Food microencapsulation of bioactive compounds: rheological and thermal characterisation of non-conventional gelling system", *Food Chemistry*, 122(2), 416-423, 2010.

[10] Doleyres Y, Lacroix C, "Technologies with free and immobilized cells for probiotic bifidobacteria production and protection", *Int Dairy J*, 15, 973-988, 2005.

[11] Grosso C.R.F, Trindade C.S.F, "Stability of free and immobilized *Lactobacillus acidophilus* and *Bifidobacterium lactis* in acidified milk and of immobilized *B. lactis* in yoghurt", *Braz J Microbiol*, 35, 151-156, 2004.

[12] Walker D.K, Gilliland S.E, "Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus"*, *J Dairy Sci*, 76, 956-961, 1993.

[13] Irvin J.L, Johnson C.G, Kopala J, "A photometric method of the determination of cholates in bile and blood", *J Biol Chem*, 153, 439-457, 1944.

[14] Aslim B, Beyatli Y, Yuksekdag Z.N, "Productions and monomer compositions of exopolysaccharides by *Lactobacillus delbrukii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains isolated from traditional home-made yoghurts and raw milk", *Int J Food Sci Technol*, 41, 973-979, 2006.

[15] Gopal S.R, Hylemon P.B, "Purification and characterization of bile salt hydrolase from *Clostridium perfringens", Journal of Lipid Research*, 29, 1079-1085, 1988.

[16] Sridevi N, Vishwe P, Prabhune A, "Hypocholesteremic effect of bile salt hydrolase from *Lactobacillus buchneri* ATCC 4005", *Food Res Int*, 42, 516-520, 2009.

[17] Yildiz G.G, Ozturk M, Aslim B "Identification of *Lactobacillus* strains from breast-fed infant and investigation of their cholesterol-reducing effects", *World J Microbiol Biotechnol*, 27(10), 2397-2306, 2011.

[18] Corzo G, Gilliland S.E, "Bile salt hydrolase activity of three strains of *Lactobacillus acidophilus*", *J Dairy Sci*, 82, 472-480, 1999.

[19] Kailasapathy K, "Microencapsulation of probiotic bacteria: technology and potential applications", *Curr. Issues Intest. Microbiol*, 3, 39-48, 2002.

[20] Gbassi G.K, Vandamme T, "Probiotic encapsulation technology: from microencapsulation to release into the gut", *Pharmaceutics*, 4, 149-163, 2012.

[21] Jones M.L, Chen H, Ouyang W, Metz T, Prakash S, "Microencapsulated genetically engineered *Lactobacillus plantarum* 80 (pCBH1) for bile acid deconjugation and its implication in lowering cholesterol" *J Biomed Biotechnol*, 1, 61-69, 2004.

[22] Tanaka H, Doesburg K, Iwasaki T, Mierau I, "Screening of lactic acid bacteria for bile salt hydrolase activity", *J Dairy Sci*, 82, 2530-2535, 1999.

[23] De Smet I, van Hoorde L, Woestyne M.V, Christiaens H, Verstraete W, "Significance of bile salt hydrolytic activities of lactobacilli", *J Appl Bacteriol*, 79, 292-301, 1995.

[24] Grill J.P, Perrin S, Schneider F, "Bile salt toxicity to some bifidobacteria strains: role of conjugated bile salt hydrolase and pH", *Can J Biochem*, 46, 878-884, 2000.