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IN VITRO CHOLESTEROL UPTAKE BY *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* ISOLATES

Małgorzata Ziarno
Warsaw University of Life Sciences – SGGW

Background. Some researchers have indicated that *Lactobacillus delbrueckii* subsp. *bulgaricus* may provide additional health benefits, reduce serum cholesterol level, for example. The aim of this study was to determine cholesterol uptake by *Lb. delbrueckii* subsp. *bulgaricus* commercial yoghurt starter isolates in artificial GIT fluids.
Material and methods. *Lb. delbrueckii* subsp. *bulgaricus* isolates were cultured in MRS broth and in artificial GIT fluids contained cholesterol at initial concentration ca. 560 µg/mL, as well as in MRS broth with cholesterol addition.
Results. All *Lb. delbrueckii* subsp. *bulgaricus* showed ability to uptake of cholesterol from MRS broth and artificial GIT fluids. The isolates incubated in artificial gastric fluid removed the minimal amounts of cholesterol in comparison to the same isolates incubated in MRS broth. Only two isolates removed significantly more cholesterol from MRS broth that from duodenal fluid. The amount of removed cholesterol from artificial duodenal fluid ranged from 20 µg/mL to 78 µg/mL. All *Lb. delbrueckii* subsp. *bulgaricus* isolates survived worse in artificial GIT fluids than in MRS broth. The viability of *Lb. delbrueckii* subsp. *bulgaricus* in GIT fluids depended on isolate.
Conclusions. These results proved that *Lb. delbrueckii* subsp. *bulgaricus* shows ability to uptake cholesterol from MRS broth and artificial GIT fluids, and the degree of cholesterol uptake depends on isolate and incubation environment. The ability of *Lb. delbrueckii* subsp. *bulgaricus* to survive in GIT varies according to the isolates and incubation environment.

Key words: cholesterol, *Lactobacillus delbrueckii* subsp. *bulgaricus*, cholesterol uptake, artificial intestinal juice, artificial duodenal juice

Corresponding author – Adres do korespondencji: Dr inż. Małgorzata Ziarno, Department of Biotechnology, Microbiology and Food Evaluation of Warsaw University of Life Sciences – SGGW, Nowoursynowska 159 C, 02-787 Warsaw, Poland, e-mail: malgorzata_ziarno@sggw.pl

INTRODUCTION

Lactobacillus delbrueckii subsp. *bulgaricus* is one of the most common lactobacilli starters used in the production of fermented milks. It is usually paired with *Streptococcus thermophilus*. The growth of *Lb. delbrueckii* subsp. *bulgaricus* in milk provides a range of amino acids that stimulate the growth of *Str. thermophilus*. *Lb. delbrueckii* subsp. *bulgaricus* uses threonine in yoghurt to produce acetaldehyde, an important aroma compound [Ott et al. 2000]. Strains of this subspecies can also produce exopolisaccharides (EPS), which influence on yoghurt texture [Grobben et al. 1995, 1998, Pigeon et al. 2002]. The genetics and the proteolytic enzymes of *Lb. delbrueckii* subsp. *bulgaricus* have been studied for years. Thus, *Lb. delbrueckii* subsp. *bulgaricus* are used as starters to produce Swiss type cheeses and Italian type cheeses [Sasaki et al. 1995, Siragusa et al. 2007].

Some researchers have indicated that *Lb. delbrueckii* subsp. *bulgaricus* may provide additional health benefits. Strains of *Lb. delbrueckii* subsp. *bulgaricus* produce bacteriocins with different activities [Simova et al. 2008]. *Lb. delbrueckii* subsp. *bulgaricus* has been shown to stimulate the secretion of cytokines by lymphocytes and monocytes in a strain-dependent manner [Meyer et al. 2007]. Hickson et al. [2007] demonstrated that consumption of a drink containing *Lb. delbrueckii* subsp. *bulgaricus* can reduce the incidence of antibiotic associated diarrhoea and *Clostridium difficile* associated diarrhoea. Additionally, Johns et al. [2007] reported the characterization of *Lb. delbrueckii* subsp. *bulgaricus* extract as a novel cytoprotective agent, which is able to attenuate TNF-α mediated induction (TNF-α plays critical role in the pathogenesis of inflammatory bowel disease).

A few researchers have indicated *in vivo* or *in vitro* that *Lb. delbrueckii* subsp. *bulgaricus* may be helpful reducing serum cholesterol level [Jaspers et al. 1984, Rasic et al. 1992, Akalin et al. 1997, Dilmi-Bouras 2006, Ziarno 2007 b, Ziarno et al. 2007]. Scientific studies demonstrated that yogurt consumption had various effects on serum cholesterol level. For example, Lin et al. [1989] showed that ingestion of commercially available *Lactobacillus* tablets, containing *Lb. bulgaricus*, did not affect serum lipoprotein concentrations. McNamara et al. [1989] demonstrated that yogurt had no effect on plasma cholesterol levels of normolipidemic males. Additionally, Thompson et al. [1982] showed significant rises in triglyceride levels in groups of healthy volunteers which were provided with supplements of yogurt daily for a 3 weeks period. Terahara et al. [2000] proved that *Lb. delbrueckii* subsp. *bulgaricus* strain 2038 can prevent the oxidation of the erythrocyte membrane *in vitro*, and the oxidation of LDL *in vivo*.

Adhesion of cholesterol to cell surface and incorporation of cholesterol into cellular membrane are the most frequently suggested mechanisms of bacteria activity on cholesterol level *in vitro* [Hosono and Tono-Oka 1995, Noh et al. 1997, Brashears et al. 1998, Usman and Hosono 1999, Kimoto et al. 2002, Tabuchi et al. 2004]. This raised an assumption that similar phenomenon may also takes place in human gastrointestinal track (GIT), where the ability to cholesterol uptake may be influenced by numerous factors, such as anaerobic condition, presence of bile salts, viability and number of bacterial cells [Gilliland et al. 1985, Grill et al. 2000, Lim et al. 2004].

The aim of this study was to evaluate the cholesterol uptake of *Lactobacillus delbrueckii* subsp. *bulgaricus* isolates in artificial GIT fluids in comparison to MRS broth.

MATERIALS AND METHODS

Sources and maintenance of cultures. Ten isolates of *Lactobacillus delbrueckii* subsp. *bulgaricus*, originated from lyophilised commercial yoghurt starter cultures, containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, were used in this study. Strains N° 1-3 were isolated from: YC-X11 starter (Chr Hansen), strains N° 4-6 were isolated from starter YC-X16 (Chr Hansen), and strains N° 7-10 from starter MYE 96 (Ezal, Rhodia Food Biolacta) The traditional microbiological plate method (MRS Agar, Merck, Germany, at 35°C/72 h under anaerobic conditions in a gas jar with anaerobic gas pak, Merck, Germany) has been applied to the isolation procedure. The bacteria were cultured twice in MRS broth (Merck, Germany) at 37°C/14 h, than were used for experiments.

Artificial gastric fluid. The artificial gastric fluid has been prepared on the basic gastric fluid and the pepsin. The basic gastric fluid has been prepared according to Clavel et al. [2004] with some modifications. It contained 4.8 g of NaCl (POCH, Poland), 1.56 g of $NaHCO_3$ (POCH, Poland), 2.2 g of KCl (POCH, Poland), and 0.22 g of $CaCl_2$ (POCH, Poland) dissolved in 1 L of distilled water. After the autoclaving at 121°C/15 min, the pH of the basic gastric fluid was adjusted to 2.4 ±0.2 using 1 M HCl, and 2 mg of pepsin (Sigma Aldrich, USA) per 50 mL of the artificial gastric fluid was added.

Artificial duodenal fluid. The artificial duodenal fluid has been prepared on the basic duodenal fluid and the enzyme complex. The basic duodenal fluid has been prepared according to Marteau et al. [1997] with some modifications. It contained 5.0 g of NaCl (POCH, Poland), 0.6 g of KCl (POCH, Poland), 0.03 g of $CaCl_2$ (POCH, Poland), and 17 g of bile salts (Merck, Germany) dissolved in 1 L of 1 mol/L $NaHCO_3$ (POCH, Poland). After the autoclaving at 121°C/15 min, the pH of the basic juice was 7.0 ±0.2 using 1 M NaOH, and the enzyme complex was added (two capsule per 50 mL of fluid). Pharmaceutical preparation called Kreon® 10 000 (Solvay Pharmaceuticals, USA) was used as source of the enzyme complex. One capsule of Kreon® 10 000 contains 150 mg of pancreatin enzymes: 10,000 F.I.P. units of lipases, 8 000 F.I.P. units of amylases, 600 F.I.P. units of proteases.

Cholesterol. Cholesterol of chemical purity >99% (Sigma-Aldrich, USA) was dissolved in 99% ethanol and Tween 80 (Merck, Germany), mixed in 3:1 ratio, and then was used as the cholesterol solution in experiments. Cholesterol has been added to the culture broth or artificial GIT fluids to reach the final concentration ca. 560 µg/mL.

The experiments. *Lb. delbrueckii* subsp. *bulgaricus* isolates were cultured in MRS broth and in artificial GIT fluids contained cholesterol at initial concentration ca. 560 µg/mL, as well as in MRS broth without cholesterol. The experiments were performed at 37°C for 5 h (in artificial gastric fluid and MRS broth) or at 37°C/6 h (in artificial duodenal fluid and MRS broth). Immediately after the adding of bacteria inoculums and at the end of experiment, the concentration of cholesterol and the number of lactobacilli were measured. The initial bacterial inoculums were 6.7-6.9 log CFU/mL of MRS broth and artificial GIT fluids.

Measurement of number of bacteria. The number of bacteria was assayed using the plate method. MRS Agar (Merck, Germany) with anaerobic incubation at 37°C/72 h was used for enumeration of lactobacilli. Each isolate were tested in five independent experiments. The viability of bacteria was expressed as percentage of log of the initial

bacterial count (log CFU/mL) in comparison to log of the final count (log CFU/mL). The viability was calculated in each experiment, then the mean and SD have been calculated.

Measurement of cholesterol uptake. It was assayed with the enzymatic diagnostic test Cholésterol RTU® (BioMérieux, France). The detection limit of the kit reagent is below 80 μg/mL and is linear up to 6970 μg/mL. Absorbance was measured with a spectrophotometer Helios Gamma (Thermo Electron Corporation, USA) at 500 nm. Before cholesterol concentration in broth was assayed the content of tubes was centrifuged (6000 rpm, 7 min, and 4°C) in order to separate bacterial cells biomass and obtain clear broth supernatant. The isolates' ability to cholesterol uptake was calculated as loss of its concentration in broth supernatant after the end of experiment. The percentage of cholesterol removed from broth during the growth of bacteria has been expressed as the percentage of the initial concentration of cholesterol.

Statistical analysis. Cholesterol uptake of *Lb. delbrueckii* subsp. *bulgaricus* isolates in artificial GIT fluids was compared with cholesterol uptake from MRS broth using the multifactor ANOVA (at the 95.0% confidence level). The statistical analysis of results was carried out with Statgraphics Plus 5.1 software.

RESULTS AND DISCUSSION

Lactobacillus delbrueckii subsp. *bulgaricus* isolates studied in the present work showed various ability to uptake of cholesterol from MRS broth and artificial GIT fluids. The results are presented in Tables 1 and 2.

Cholesterol uptake in artificial gastric fluid. Initial cholesterol concentration in the bacteria cultures ranged from 549 μg/mL for isolates No 8 & 9 to 582 μg/mL for isolates No 1, 2, and 3. The isolates incubated in artificial gastric fluid removed the minimal amounts of cholesterol in comparison to the same isolates incubated in MRS broth (Table 1). All strains caused significantly reduction in the amount of cholesterol level during the 5 h incubation in MRS broth (p-value = 0.0001), as well as during the 5 h incubation in gastric fluid (p-value = 0.0262). The amounts of cholesterol removed from gastric fluid or MRS broth statistically significantly depended on isolates (p-value = 0.0007). Isolate No 4 removed 3 μg/mL and 25 μg/mL of cholesterol from gastric fluid and MRS broth, respectively. In comparison, isolate No 6 removed 31 μg/mL and 85 μg/mL of cholesterol from gastric fluid and MRS broth, respectively. The percent of cholesterol removed from artificial gastric fluid ranged from 0.5% for isolate No 4 to 5.6% for isolate No 6. For the same incubation period in MRS broth, the amounts of removed cholesterol ranged from 4.5% for isolate No 4 to 15.4% for isolate No 6.

Cholesterol uptake in artificial duodenal fluid. Results from the comparison of *Lb. delbrueckii* subsp. *bulgaricus* isolates for cholesterol uptake in artificial duodenal fluid and MRS broth are summarized in Table 2. The studied isolates also exhibited significant variation with regard to cholesterol uptake in artificial duodenal fluid. The amount of cholesterol removed from artificial duodenal fluid ranged from 20 μg/mL for isolate No 3 (3.3% of initial cholesterol level) to 78 μg/mL for isolate No 6 (13.3% of initial cholesterol level). The amount of removed cholesterol from MRS broth ranged from 34 μg/mL for isolate No 4 (5.7% of initial cholesterol level) to 86 μg/mL for isolate No 6 (14.6% of initial cholesterol level). The result showed that the amount

Table 1. The uptake of cholesterol by *Lb. delbrueckii* subsp. *bulgaricus* isolates in artificial gastric fluid and in MRS broth

Isolate no	Initial concentration of cholesterol µg/mL	In artificial gastric fluid			In MRS broth		
		concentration of cholesterol µg/mL		percentage of removed cholesterol	concentration of cholesterol µg/mL		percentage of removed cholesterol
		after 5 h	difference		after 5 h	difference	
1	582 ±12	574 ±11	8 ±1 bc	1.4 ±0.2 bc	533 ±16	50 ±16 bc	8.5 ±2.6 b
2	582 ±12	572 ±12	10 ±1 c	1.8 ±0.1 bc	510 ±17	72 ±9 de	12.4 ±1.6 c
3	582 ±12	579 ±9	4 ±3 a	0.6 ±0.4 a	524 ±17	58 ±16 bcd	10.0 ±2.7 bc
4	551 ±33	548 ±33	3 ±1 a	0.5 ±0.2 a	526 ±32	25 ±9 a	4.5 ±1.7 a
5	551 ±33	529 ±36	21 ±5 d	3.9 ±1.1 d	508 ±29	43 ±8 b	7.8 ±1.2 b
6	551 ±33	520 ±31	31 ±4 e	5.6 ±0.7 e	466 ±32	85 ±8 e	15.4 ±1.6 d
7	552 ±37	533 ±37	20 ±5 d	3.6 ±1.0 d	495 ±42	58 ±18 bcd	10.5 ±3.4 bc
8	549 ±35	538 ±36	12 ±2 c	2.1 ±0.6 c	479 ±39	70 ±19 de	12.8 ±3.6 cd
9	549 ±35	543 ±33	6 ±4 ab	1.1 ±0.7 ab	483 ±25	66 ±12 cd	12.0 ±1.6 c
10	559 ±20	547 ±21	12 ±1 c	2.2 ±0.3 c	502 ±9	57 ±13 bcd	10.1 ±1.9 bc

Results are expressed as mean ±standard deviation, n = 5.

abc... – different letters in the same column indicate significant differences between treatments with LSD test ($p < 0.05$).

Table 2. The uptake of cholesterol by *Lb. delbrueckii* subsp. *bulgaricus* isolates in artificial duodenal fluid and in MRS broth

Isolate no	Initial concentration of cholesterol µg/mL	In artificial duodenal fluid			In MRS broth		
		concentration of cholesterol µg/mL		percentage of removed cholesterol	concentration of cholesterol µg/mL		percentage of removed cholesterol
		after 6 h	difference		after 6 h	difference	
1	596 ±19	571 ±27	25 ±9 ab	4.2 ±1.7 ab	536 ±27	59 ±12 bcd	10.0 ±2.0 bc
2	596 ±18	572 ±21	24 ±5 ab	4.0 ±0.8 ab	517 ±21	79 ±10 ef	13.2 ±1.6 de
3	596 ±20	576 ±22	20 ±9 a	3.3 ±1.5 a	549 ±26	46 ±8 ab	7.8 ±1.5 ab
4	591 ±10	555 ±10	36 ±5 bc	6.1 ±0.8 bc	557 ±25	34 ±18 a	5.7 ±3.1 a
5	591 ±9	522 ±17	69 ±17 d	11.6 ±2.8 d	541 ±12	50 ±11 bc	8.5 ±1.8 abc
6	591 ±8	512 ±16	78 ±12 d	13.3 ±2.1 d	504 ±10	86 ±7 f	14.6 ±1.2 e
7	593 ±10	549 ±13	44 ±5 c	7.4 ±0.8 c	542 ±21	52 ±12 bcd	8.7 ±2.1 bc
8	597 ±4	573 ±8	24 ±8 ab	4.0 ±1.3 ab	518 ±15	78 ±15 ef	13.1 ±2.5 de
9	597 ±5	527 ±19	70 ±19 d	11.7 ±3.1 d	532 ±14	65 ±12 cde	10.9 ±5.0 cd
10	600 ±6	527 ±17	73 ±16 d	12.2 ±2.6 d	534 ±16	67 ±17 de	11.1 ±2.9 cd

Results are expressed as mean ± standard deviation, n = 5.

abc... – different letters in the same column indicate significant differences between treatments with LSD test ($p < 0.05$).

of removed cholesterol depended on isolate (p-value = 0.0001), however only two isolates (No 2 & 8) removed significantly more cholesterol from MRS broth that from duodenal fluid (p-value = 0.0001). Eight of ten studied isolates demonstrated similar cholesterol uptake from duodenal fluid as from MRS broth.

Dairy or pharmaceutical *Lb. acidophilus* isolates studied in previous studies were characterised by high ability to uptake of cholesterol during growth in MRS broth compared to other studied lactic acid bacteria cultures [Ziarno 2007 b, 2008, Ziarno et al. 2007]. Previous studied *Lb. acidophilus* isolates removed much more cholesterol from artificial duodenal fluid (0.6-2.9%) than from gastric fluid (3.8-13.5%), but much less than from MRS broth (8.4-16.8%) [Ziarno 2008]. As for study regarding assessment of *Lb. delbrueckii* subsp. *bulgaricus* isolates' ability to uptake cholesterol there is no information in literature indicating that cholesterol uptake by this subspecies from artificial GIT fluids was studied. The results of present study demonstrated that *Lb. delbrueckii* subsp. *bulgaricus* isolates have similar ability to uptake cholesterol than previous studied *Lb. acidophilus* isolates.

In the present work, *Lb. delbrueckii* subsp. *bulgaricus* isolates showed ability to remove cholesterol during the incubation in GIT fluids. The degree of cholesterol uptake depended on isolate and incubation environment. The higher cholesterol uptake was observed during the incubation in MRS broth. The lowest reduction of cholesterol level was observed in artificial gastric fluid. From artificial duodenal fluid *Lb. delbrueckii* subsp. *bulgaricus* isolates removed more cholesterol than from artificial gastric fluid. It could suggests that the incubation environment with neutral acidity is more beneficial for cholesterol uptake than acidic environment. It is confirmation of observations made by Lin and Chen [2000] and Ziarno [2008] towards *Lb. acidophilus* isolates.

Present study on the assimilation of cholesterol *in vitro* by *Lb. delbrueckii* subsp. *bulgaricus isolates* is in agreement with earlier studies carried out by Rasic et al. [1992], and Ziarno et al. [2007]. Rasic et al. [1992] studied the assimilation of cholesterol by some culture of lactic acid bacteria and bifidobacteria in MRS broth during 18 h culture in 37°C. The researchers demonstrated significant difference in assimilation of cholesterol between three strains of *Lb. delbrueckii* subsp. *bulgaricus*: LB1, LB2, and LB3, which assimilated 276 μg/mL, 102 μg/mL, and 123 μg/mL, respectively. Cited researchers obtained high values of removed cholesterol, but they incubated bacteria for 18 h, and in the present study the incubation period was 5-6 h. Ziarno et al. [2007] studied cholesterol assimilation by selected commercial yoghurt starter cultures during 24 h incubation in MRS broth. Commercial starter cultures (consisting of *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) showed different ability to uptake cholesterol from MRS broth, but the amount of removed cholesterol did not exceed 189 μg/mL (i.e. 27% of its initial contents).

It is well known that the uptake of cholesterol by some lactic acid bacteria takes place in the presence of bile salts and it is resulted partially from cholesterol co-precipitation together with deconjugated bile salts. It means that uptake of cholesterol in the presence of bile salts is resulted not only from its removal by bacteria cells [Klaver and Meer 1993, Gopal et al. 1996, Brashears et al. 1998]. Dilmi-Bouras [2006] also demonstrated that *Lb. delbrueckii* subsp. *bulgaricus* strains have ability to assimilate cholesterol *in vitro* in MRS broth with and without bile salts addition (0.0 and 0.3%). Few researchers indicated that pH value in human intestinal is neutral and the cholesterol co-precipitation together with deconjugated bile salts is impossible [Brashears et al. 1998, Lin and Chen 2000, Ziarno 2004, Liong and Shah 2005 b].

Viability in artificial gastric fluid. In present study, all *Lb. delbrueckii* subsp. *bulgaricus* isolates survived worse in artificial gastric fluid than in MRS broth (Table 3). The viability of *Lb. delbrueckii* subsp. *bulgaricus* in artificial gastric fluid depended on isolate (p-value = 0.0001). The initial number of bacteria ranged from 6.8 log CFU/mL to 6.9 log CFU/mL, and decreased to 4.0-5.2 log CFU/mL after 5 h incubation in artificial gastric fluid. Viability was 59.6-78.2%. Simultaneously, the final number of bacteria in MRS broth was 6.6-6.9 log CFU/mL, and viability ranged from 96.8% to 101.5%.

Table 3. The viability of *Lb. delbrueckii* subsp. *bulgaricus* isolates in artificial gastric fluid and in MRS broth

Isolate no	Initial number of bacteria log CFU/mL	In artificial gastric fluid			In MRS broth		
		final number of bacteria log CFU/mL		viability %	final number of bacteria log CFU/mL		viability %
		after 5 h	difference		after 5 h	difference	
1	6.9 ±0.2	4.4 ±0.1	2.4 ±0.3 ef	64.6 ±3.0 bc	6.8 ±0.1	0.1 ±0.3 abc	98.9 ±3.8 abc
2	6.7 ±0.1	4.3 ±0.1	2.4 ±0.1 ef	64.4 ±1.2 bc	6.8 ±0.2	–0.1 ±0.2 ab	100.9 ±3.1 bc
3	6.7 ±0.1	5.2 ±0.1	1.5 ±0.1 a	78.2 ±1.6 f	6.7 ±0.1	0.0 ±0.1 ab	100.3 ±2.0 bc
4	6.7 ±0.1	4.6 ±0.1	2.1 ±0.1 d	68.2 ±1.9 d	6.6 ±0.1	0.2 ±0.2 bc	97.6 ±2.5 ab
5	6.8 ±0.2	4.5 ±0.2	2.3 ±0.3 de	66.6 ±3.5 cd	6.6 ±0.1	0.2 ±0.2 c	96.8 ±2.4 a
6	6.8 ±0.2	5.2 ±0.1	1.6 ±0.2 ab	76.0 ±2.9 f	6.7 ±0.1	0.1 ±0.2 abc	98.3 ±2.4 abc
7	6.7 ±0.1	4.0 ±0.1	2.7 ±0.1 g	59.6 ±1.4 a	6.7 ±0.1	0.0 ±0.2 ab	100.6 ±2.3 bc
8	6.7 ±0.1	4.2 ±0.1	2.5 ±0.2 fg	62.1 ±2.1 ab	6.7 ±0.1	0.0 ±0.2 abc	100.0 ±3.2 abc
9	6.8 ±0.1	5.0 ±0.1	1.8 ±0.2 bc	73.0 ±2.6 e	6.8 ±0.1	0.0 ±0.1 ab	100.3 ±0.1 bc
10	6.8 ±0.1	4.9 ±0.2	1.9 ±0.2 c	72.4 ±2.4 e	6.9 ±0.1	–0.1 ±0.1 a	101.5 ±0.1 c

Results are expressed as mean ± standard deviation, n = 5.

abc... – different letters in the same column indicate significant differences between treatments with LSD test ($p < 0.05$).

Viability in artificial duodenal fluid. All *Lb. delbrueckii* subsp. *bulgaricus* isolates survived significantly better in MRS broth than in artificial duodenal fluid (p-value = 0.0001). The initial number of bacteria ranged from 6.6 log CFU/mL to 6.9 log CFU/mL (Table 4). The viability of *Lb. delbrueckii* subsp. *bulgaricus* depended on the isolates (p-value = 0.0001). After 6 h of incubation the population of *Lb. delbrueckii* subsp. *bulgaricus* has been reduced to 3.3-6.2 log CFU/mL, and viability was 48.2--92.0%. Simultaneously, the final number of bacteria in MRS broth was 6.7-6.8 log CFU/mL, and viability ranged from 98.3% to 101.6%.

Lb. delbrueckii subsp. *bulgaricus*, one of the two most important organisms used in yogurt manufacture, has been reported in scientific literature to have poor survival in the gastrointestinal tract because of its low tolerance for bile salts, low resistance to acid pH, and rather selective requirements for sugars [Conway et al. 1987, Marteau et al. 1997, Taranto et al. 2003]. Nevertheless, some researchers demonstrated that this organism survives passage through the intestinal tract of humans [Akalin et al. 1997, Marteau

Table 4. The viability of *Lb. delbrueckii* subsp. *bulgaricus* isolates in artificial duodenal fluid and in MRS broth

Isolate no	Initial number of bacteria log CFU/mL	In artificial duodenal fluid			In MRS broth		
		final number of bacteria log CFU/mL		viability %	final number of bacteria log CFU/mL		viability %
		after 6 h	difference		after 6 h	difference	
1	6.7 ±0.2	6.2 ±0.3	0.5 ±0.1 a	92.0 ±1.4 g	6.8 ±0.1	-0.1 ±0.3 a	101.6 ±4.2 a
2	6.7 ±0.1	5.3 ±0.3	1.4 ±0.2 d	79.7 ±3.3 c	6.8 ±0.1	-0.1 ±0.1 a	101.5 ±2.2 a
3	6.8 ±0.1	6.0 ±0.1	0.8 ±0.1 b	88.0 ±1.1 ef	6.7 ±0.1	0.1 ±0.1 a	98.8 ±1.9 a
4	6.8 ±0.2	3.3 ±0.4	3.5 ±0.3 f	48.2 ±4.8 a	6.7 ±0.1	0.0 ±0.2 a	99.4 ±2.2 a
5	6.7 ±0.3	4.1 ±0.1	2.6 ±0.2 e	61.0 ±2.0 b	6.7 ±0.1	0.0 ±0.3 a	100.1 ±4.3 a
6	6.6 ±0.1	5.8 ±0.1	0.8 ±0.1 b	87.4 ±1.5 e	6.7 ±0.1	0.0 ±0.2 a	100.3 ±3.1 a
7	6.8 ±0.2	6.1 ±0.3	0.6 ±0.1 ab	90.8 ±2.0 fg	6.7 ±0.2	0.0 ±0.3 a	99.5 ±4.4 a
8	6.9 ±0.1	5.7 ±0.2	1.1 ±0.2 c	83.7 ±3.1 d	6.7 ±0.1	0.1 ±0.2 a	98.3 ±2.8 a
9	6.7 ±0.1	6.1 ±0.2	0.7 ±0.2 ab	90.2 ±2.5 efg	6.7 ±0.1	0.0 ±0.3 a	100.1 ±5.1 a
10	6.7 ±0.1	6.1 ±0.2	0.7 ±0.1 ab	90.2 ±1.9 efg	6.8 ±0.1	0.0 ±0.2 a	100.7 ±3.4 a

Results are expressed as mean ± standard deviation, n = 5.
abc... – different letters in the same column indicate significant differences between treatments with LSD test ($p < 0.05$).

et al. 1997, Mater et al. 2005, Elli et al. 2006]. Marteau et al. [1997] demonstrated *in vitro* the differences among bacterial species in their sensitivity to gastric and intestinal secretions. The survival of lactic acid bacteria strains in GIT model was investigated under two different conditions in the small intestine: simulation of physiological secretion of bile and low bile secretion. Researchers showed that reductions in viability were significantly different between the bacterial species and depended on bile concentration. Elli et al. [2006] and Mater et al. [2005] also proved *in vivo* experiments good survival of yogurt bacteria in the human gut. The study carried out by Elli et al. [2006] included 20 healthy male and female which ate 125 g of commercial yogurt twice a day for 1 week. The daily intake of bacteria from yogurt, containing 2.4×10^7 CFU/g of *Lb. delbrueckii* subsp. *bulgaricus* and 2.0×10^8 CFU/g of *Str. thermophilus*, was about 6×10^9 CFU of *Lb. delbrueckii* subsp. *bulgaricus* and 5×10^{10} CFU of *Str. thermophilus*. Yogurt bacteria were found in faecal samples, which were obtained at the beginning of each trial (zero time) and after 2 and 7 days. Mater et al. [2005] also recovered viable cells of *Lb. delbrueckii* subsp. *bulgaricus* from faeces of healthy volunteers fed 375 g of yogurt per day.

The previous study towards the ability of lactic acid bacteria cells to survive in artificial GIT fluids showed differences amongst the species and genera of bacteria (*Lb. acidophilus*, *Lb. casei*, *Lb. plantarum*, *Lb. rhamnosus*, bifidobacteria, *Lactococcus lactis*, and *Str. thermophilus*) [Ziarno 2007 a, 2008]. Present study confirmed that *Lb. delbrueckii* subsp. *bulgaricus* isolates possessed some survival ability in artificial GIT fluids. The results suggested that *Lb. delbrueckii* subsp. *bulgaricus* isolates survived

in GIT fluids and could reduce cholesterol level. Even if the bacteria had not possibility to grow in artificial GIT fluids, the cholesterol uptake by bacteria cells took place. These results are compatible with the previous observations made by Brashears and Gilliland [1995], Tabuchi et al. [2004], Liong and Shah [2005 a], and Ziarno [2008].

Cholesterol uptake and survival during passage through the human GIT are generally considered a key feature for probiotics to preserve their expected health-promoting effects [Bezkorovainy 2001]. Nevertheless, the application of the term "probiotic" to subspecies *Lb. delbrueckii* subsp. *bulgaricus* is still under discussion [Weid et al. 2001, Guarner et al. 2005]. The present study confirmed that *Lb. delbrueckii* subsp. *bulgaricus* isolates possessed ability to survive in artificial GIT fluids, and it could also remove some significant amounts of cholesterol in these conditions.

CONCLUSIONS

1. *Lb. delbrueckii* subsp. *bulgaricus* isolates shows ability to uptake cholesterol from MRS broth and artificial GIT fluids. The degree of cholesterol uptake depends on isolate and incubation environment.

2. All isolates of *Lb. delbrueckii* subsp. *bulgaricus* remove more cholesterol from MRS broth and from artificial duodenal fluid than from artificial gastric fluid, but only two isolates remove significantly more cholesterol from MRS broth that from duodenal fluid.

3. The ability of *Lb. delbrueckii* subsp. *bulgaricus* cells to survive in GIT varies according to the isolates.

4. All *Lb. delbrueckii* subsp. *bulgaricus* isolates survive worse in GIT fluids than in MRS broth.

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USUWANIE CHOLESTEROLU W WARUNKACH *IN VITRO* PRZEZ IZOLATY *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS*

Wprowadzenie. Niektórzy badacze dowodzą, że *Lb. delbrueckii* subsp. *bulgaricus* mogą wykazywać korzystne działanie na organizm człowieka, na przykład obniżać poziom cholesterolu w serum krwi. Celem badań było określenie zdolności izolatów *Lactobacillus delbrueckii* subsp. *bulgaricus* do usuwania cholesterolu w warunkach modelowych soków żołądkowego i trzustkowego.

Materiały i metody. *Lb. delbrueckii* subsp. *bulgaricus* były inkubowane w bulionie MRS oraz modelowych sokach żołądkowym i trzustkowym zawierających dodatek 560 µg cholesterolu w 1 cm³.
Wyniki. Badane *Lb. delbrueckii* subsp. *bulgaricus* wykazały się różną zdolnością do usuwania cholesterolu z bulionu MRS oraz modelowych soków żołądkowego i trzustkowego. Izolaty inkubowane w modelowym soku żołądkowym usuwały minimalne ilości cholesterolu w porównaniu z inkubacją w bulionie MRS. Jedynie dwa izolaty usunęły istotnie więcej cholesterolu z bulionu MRS niż z modelowego soku trzustkowego. Ilość usuniętego cholesterolu z modelowego soku trzustkowego wynosiła od 20 µg/cm³ do 78 µg/cm³. Wszystkie *Lb. delbrueckii* subsp. *bulgaricus* przeżyły gorzej warunki modelowych soków żołądkowego lub trzustkowego niż w bulionie MRS. Przeżywalność *Lb. delbrueckii* subsp. *bulgaricus* w modelowych sokach żołądkowym lub trzustkowym zależała od izolatu.
Wnioski. Wyniki dowiodły, że *Lb. delbrueckii* subsp. *bulgaricus* wykazują zdolność do usuwania cholesterolu z bulionu MRS i modelowych soków trawiennych, ale ilość usuwanego cholesterolu zależy od izolatu i warunków środowiskowych. Zdolność komórek *Lb. delbrueckii* subsp. *bulgaricus* do przeżywania w modelowych sokach trawiennych także zależy od izolatu i środowiska.

Słowa kluczowe: cholesterol, *Lactobacillus delbrueckii* subsp. *bulgaricus*, usuwanie cholesterolu, modelowy sok żołądkowy, modelowy sok trzustkowy

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