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IN VITRO CHOLESTEROL UPTAKE BY *LACTOBACILLUS ACIDOPHILUS* ISOLATES

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Abstract. The aim of this study was to evaluate cholesterol uptake by *Lb. acidophilus* isolates in artificial gastric and duodenal fluids. Studied *Lb. acidophilus* isolates showed various abilities to uptake cholesterol from MRS broth and artificial GIT fluids. From artificial duodenal fluid *Lb. acidophilus* isolates removed more cholesterol (from 0.6% to 2.9%) than from gastric fluid (from 3.8% to 13.5%). Even if bacteria had no possibility to grow in artificial GIT fluids, the cholesterol uptake by bacteria cells took place. *Lb. acidophilus* isolates survived worse in simulated gastric or duodenal fluids than in MRS broth. The initial number of bacteria ranged from 6.2 log CFU/mL to 7.1 log CFU/mL, and viability in artificial gastric fluid was 60.6-81.9% of log of the initial number of bacteria. Viability in artificial duodenal fluid was 35.1-87.3%.

Key words: cholesterol, *Lactobacillus acidophilus*, cholesterol uptake, simulated intestinal juice, simulated duodenal juice

INTRODUCTION

Lactobacillus acidophilus is the most commonly probiotic used commercially in dairy industry in the production of probiotic fermented milks, yoghurts especially. Some research has indicated that *Lb. acidophilus* may provide additional health benefits. Thus *Lb. acidophilus* is included in the pharmaceutical supplements which are often recommended after a course of antibiotic therapy.

Researchers have indicated that *Lb. acidophilus* may be helpful reducing serum cholesterol levels or remove cholesterol from growth medium [Gilliland et al. 1985, Rasic et al. 1992, Lin and Chen 2000, Saito 2004]. The *in vitro* experiments have demonstrated that many LAB have an ability to reduce cholesterol level in the growth medium containing bile salts. The ability to cholesterol uptake may be influenced by numerous factors, such as kind of medium, presence of bile salts, viability and number of bacterial cells.

The ability to *in vitro* uptake of cholesterol level in model culture media has been yet shown for numerous strains of *Lactobacillus* genera, such as *Lb. acidophilus*, *Lb.*

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delbrueckii subsp. *bulgaricus*, *Lb. casei*, *Lb. gasseri*, and *Lb. amylovorus* [Gilliland et al. 1985, Nielson and Gilliland 1985, Gilliland and Walker 1989, Rasic et al. 1992, Walker and Gilliland 1993, Buck and Gilliland 1994, Brashears et al. 1998, Lin and Chen 2000]. Adhesion of cholesterol to cell surface and incorporation of cholesterol into cellular membrane are the most frequently suggested mechanisms of LAB's activity on cholesterol level [Hosono and Tono-Oka 1995, Brashears et al. 1998]. This raised an assumption that similar phenomenon may also takes place in human GIT.

The aim of this study was to evaluate the cholesterol uptake of *Lb. acidophilus* isolates in artificial gastric and duodenal fluids.

MATERIALS AND METHODS

Sources and maintenance of cultures. Ten isolates of *Lactobacillus acidophilus* were used in this study. The traditional microbiological plate methods (ROGOSA Agar, Merck, Germany, at 35°C/72 h under anaerobic conditions in a gas jar with anaerobic gas pak, Merck, Germany) have been applied to the isolation procedure. The isolates No 1-6 originated from commercial dairy starter cultures and the isolates No 7-10 have been isolated from market pharmaceutical supplements. The bacteria were cultured twice in MRS broth [Merck, Germany] at 37°C/12 h, then 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, 8000 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. 500 µg/mL. This concentration is comparable to cholesterol content in dairy products. For example, 3.2% milk contains ca. 140 µg of cholesterol per 1 g, 12% cream contains approx. 390 µg/1 g, 2% natural yoghurt contains 110-80 µg/1 g, cheddar cheese contains ca. 1.000 µg/1 g, and butter contains ca. 2.300 µg/1 g. It is known that in coronary heart disease (CHD)

the low-cholesterol diet [below 300 µg] is recommended. In addition, total cholesterol level in human blood should not exceed 220 µg/mL.

The experiments. *Lb. acidophilus* isolates were cultured in MRS broth and in artificial GIT fluids contained cholesterol at initial concentration ca. 500 µg/mL, as well as in the control media without cholesterol. The experiments were performed at 37°C for 5 h (in simulated gastric fluid and MRS broth) or at 37°C/6 h (in simulated duodenal fluid and MRS broth). Immediately after adding of bacteria inoculums and at the end of the experiment, the concentration of cholesterol and the number of lactobacilli were measured. The initial bacterial inoculums were 5.9-6.9 log CFU/mL of culture broth, gastric fluid, and duodenal fluid.

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 was 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 were 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 6.970 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 cultures' 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 was expressed as the percentage of the initial concentration of cholesterol.

Statistical analysis. Cholesterol uptake of *Lb. acidophilus* isolates in artificial gastric and duodenal fluid 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 4.1 software.

RESULTS AND DISCUSSION

Lb. acidophilus isolates studied in the present work showed various ability to uptake cholesterol from MRS broth and artificial GIT fluids. The results are presented in Tables 1 and 3.

Cholesterol uptake in artificial gastric fluid. Initial cholesterol concentration in the bacteria cultures ranged from 506 µg/mL for isolates No 5 & 6 to 541 µg/mL for isolates No 7-10. Isolates suspended in artificial gastric fluid removed the minimal amounts of cholesterol (Table 1). All strains caused significantly higher reduction in the amount of cholesterol during the 5 h growth period in MRS broth than during the 5 h growth period in gastric fluid. The percentage of removed cholesterol from artificial gastric fluid ranged from 0.6% for isolate No 10 to 2.9% for isolate No 6. For the same culture time the amounts of cholesterol uptake from MRS broth ranged from 8.4% for isolate No 5 to 16.1% for isolate No 3. Isolates No 10 and 6 removed respectively 47 µg/mL and 73 µg/mL of cholesterol from MRS broth.

Table 1. The uptake of cholesterol by *Lb. acidophilus* 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	513 ±49	507 ±50	6 ±2	1.2 ±0.5	446 ±49	67 ±2	13.2 ±1.2
2	515 ±52	508 ±52	7 ±1	1.3 ±0.2	442 ±51	73 ±4	14.2 ±1.3
3	513 ±48	509 ±49	4 ±1	0.8 ±0.2	431 ±45	82 ±7	16.1 ±1.2
4	513 ±48	500 ±48	13 ±3	2.5 ±0.6	449 ±50	64 ±7	12.5 ±1.9
5	506 ±38	501 ±37	6 ±2	1.1 ±0.2	464 ±41	42 ±6	8.4 ±1.6
6	506 ±37	491 ±35	15 ±3	2.9 ±0.5	432 ±39	73 ±5	14.6 ±1.6
7	541 ±84	529 ±83	12 ±3	2.3 ±0.4	463 ±66	78 ±21	14.3 ±2.2
8	541 ±84	533 ±84	8 ±2	1.5 ±0.4	484 ±72	57 ±13	10.5 ±1.1
9	541 ±84	534 ±84	7 ±1	1.4 ±0.3	482 ±80	59 ±6	11.0 ±1.2
10	541 ±84	538 ±84	3 ±1	0.6 ±0.2	494 ±77	47 ±9	8.7 ±1.1

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

Table 2. The viability of *Lb. acidophilus* 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	6.4 ±0.5	4.5 ±0.7	1.9 ±0.3	70.0 ±5.8	6.4 ±0.6	0.1 ±0.2	99.1 ±2.4
2	6.6 ±0.5	4.7 ±0.7	1.9 ±0.6	71.8 ±9.3	6.6 ±0.3	0.1 ±0.2	98.8 ±2.7
3	6.9 ±0.2	5.4 ±0.5	1.5 ±0.4	78.8 ±5.4	6.8 ±0.4	0.0 ±0.2	100.0 ±3.2
4	6.3 ±0.4	4.3 ±0.8	2.0 ±0.5	67.6 ±8.9	6.3 ±0.2	0.0 ±0.1	100.4 ±1.7
5	6.4 ±0.2	3.9 ±0.6	2.5 ±0.6	60.6 ±9.4	6.5 ±0.3	0.0 ±0.1	100.0 ±2.2
6	7.1 ±0.8	5.2 ±0.6	1.9 ±0.5	72.8 ±5.3	7.1 ±0.4	–0.1 ±0.2	101.5 ±2.6
7	6.4 ±0.3	5.2 ±0.1	1.2 ±0.3	81.9 ±4.5	6.5 ±0.5	0.0 ±0.2	100.6 ±3.5
8	6.2 ±0.5	4.6 ±0.5	1.6 ±0.4	73.8 ±5.9	6.4 ±0.5	0.0 ±0.2	100.3 ±3.3
9	6.2 ±0.4	5.0 ±0.4	1.2 ±0.6	81.1 ±8.0	6.6 ±0.3	–0.3 ±0.3	104.2 ±5.0
10	6.4 ±0.4	4.7 ±0.5	1.8 ±0.3	72.5 ±4.7	6.7 ±0.4	0.1 ±0.3	98.8 ±4.5

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

Table 3. The uptake of cholesterol by *Lb. acidophilus* 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	513 ±49	448 ±42	65 ±11	12.6 ±1.6	437 ±47	76 ±6	14.9 ±1.4
2	515 ±52	495 ±53	20 ±7	3.8 ±1.4	442 ±49	73 ±6	14.3 ±1.1
3	513 ±48	472 ±50	41 ±9	8.0 ±2.1	426 ±40	86 ±13	16.8 ±1.9
4	513 ±48	455 ±54	57 ±12	11.3 ±3.0	443 ±49	70 ±8	13.7 ±2.1
5	506 ±38	482 ±31	24 ±8	4.7 ±1.2	455 ±45	52 ±9	10.3 ±2.5
6	506 ±37	438 ±37	68 ±6	13.5 ±1.4	439 ±36	67 ±6	13.3 ±1.3
7	541 ±84	495 ±81	46 ±6	8.6 ±1.0	456 ±76	85 ±10	15.9 ±1.4
8	541 ±84	499 ±83	42 ±2	7.9 ±0.9	481 ±72	60 ±14	11.1 ±1.0
9	541 ±84	484 ±83	57 ±12	10.7 ±2.7	475 ±82	66 ±9	12.4 ±2.3
10	541 ±84	506 ±86	35 ±5	6.7 ±1.5	492 ±78	49 ±11	9.0 ±1.7

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

Cholesterol uptake in artificial duodenal fluid. Results from the comparison of *Lb. acidophilus* isolates for cholesterol uptake in simulated duodenal fluid and MRS broth are summarized in Table 3. The studied isolates also exhibited significant variation with regard to cholesterol uptake in simulated duodenal fluid. Isolates No 2, 3, 5, 7, and 8 (50% of studied isolates) removed significantly less cholesterol from artificial duodenal fluid than from MRS broth. The amount of removed cholesterol from artificial duodenal fluid ranged from 20 µg/mL for isolate No 2 to 46 µg/mL for isolate No 7. They removed 3.8-8.6% of cholesterol and 11.1-16.8% from artificial duodenal fluid and MRS broth, respectively. All other isolates removed the same amounts of cholesterol from simulated duodenal fluid compared to MRS broth. The percentage of removed cholesterol from artificial duodenal fluid ranged from 6.7% for isolate No 10 to 13.5% ±for isolate No 6.

As for study regarding assessment of *Lb. acidophilus* isolates' ability to uptake cholesterol there is no information in literature indicating that cholesterol uptake by LAB from simulated GIT fluids was studied. The monocultures of *Lb. acidophilus* studied in previous studies, being dairy starter cultures, were characterized by high ability to uptake of cholesterol during growth in MRS broth compared to other studied LAB strains [Ziarno 2007 b, Ziarno et al. 2007]. Value of this removal was 24.5% and 52.4% (the initial cholesterol content was 700 µg/mL, the cultures grown at 37°C/24 h), despite of the fact that the number of bacterial cells in these cultures did not differ from numbers of bacteria in other cultures.

Rasic et al. [1992] observed ability of three *Lb. acidophilus* strains to uptake cholesterol in MRS broth during 18 h culture in 37°C. The uptake value for *Lb. acidophilus* strains was from 177 g to 225 µg/mL of medium. Results of other studies confirmed cholesterol uptake by *Lb. acidophilus* strains from culture broth. But the culture broth enriched with bile salts, single (for example sodium taurocholate) or mixtures of conjugated or deconjugated bile acid salts (for example oxgall) were used in the most of published studies. Lin and Chen [2000] studied cholesterol level reduction ability of six strains of *Lb. acidophilus* during culture in three different media: with addition of oxgall (mixture of conjugated and deconjugated bile salts), cholic acid (source of deconjugated bile salts), or taurocholic acid (source of conjugated bile salts). All media contained 2% PPLO as the source of cholesterol and 0.5% oxgall, cholic acid, or taurocholic acid. The cholesterol contents in media ranged from 52 to 57 µg/mL. The studied strains removed from 20% to 57% cholesterol in the presence of oxgall, from 43% to 71% cholesterol in the presence of cholic acid, and 11-52% cholesterol in the presence of taurocholic acid. The uptake of cholesterol by LAB resulted probably from its precipitation together with deconjugated bile salts but not from its removal by bacteria cells [Walker and Gilliland 1993, Gopal et al. 1996, Brashears et al. 1998]. Klaver and Meer [1993] observed the coprecipitation of cholesterol, which occurred only in the presence of deconjugated bile salts at pH values lower than 6.0. On this basis they concluded that reduction of cholesterol level is a result of reduced solubility of deconjugated bile acids and coprecipitation of cholesterol with them. The pKas of taurine- and glycine-conjugated bile salts, and of unconjugated bile salts, are pH 1.9, 3.9, and 5.0, respectively [Dashkevicz and Feighner 1989]. Ahn et al. [2003] demonstrated that some strains of *Lb. acidophilus* could precipitated more than 50% of cholesterol in the medium containing taurodeoxycholate or taurochenodeoxycholate. But it seems that coprecipitation is not likely to take place *in vivo* since the pH in the small intestine it is higher than 6.0 [Lin and Chen 2000]. The duodenum is the first section of the small intestine. The duodenal fluid contains many enzymes (proteolytic enzymes, glucoamylases, oligo-1,6-glucosidases, saccharases, maltases, lactases and lipases) produced by the pancreas, and bile salts secreted by the liver. The pH of pancreatic fluid is 7.0-8.7 and the pH of the duodenal fluid is 6.5-7.5. Additionally, in the duodenum, there are Brunner glands secreting the mucus at pH 8.3--9.3, that neutralize the acidity of stomach fluid and protect the duodenum before low pH of the content inflowing from the stomach.

Lb. acidophilus isolates studied in this research showed ability to remove the cholesterol during the incubation in gastric and intestinal juices. The degree of cholesterol uptake depended on the environment (Table 1 and 3). The higher cholesterol uptake was observed during the culture in MRS broth which did not contain bile salts. The lowest reduction of cholesterol content was observed in artificial gastric fluid. From artificial duodenal fluid *Lb. acidophilus* isolates removed more cholesterol than from gastric fluid. It means that the environment with neutral acidity is more beneficial for cholesterol uptake than acidic environment. It is confirmation of suggestion made by Lin and Chen [2000].

Viability in artificial gastric fluid. In this work, *Lb. acidophilus* isolates survived worse in simulated gastric fluid than in MRS broth (Table 2). The initial number of bacteria ranged from 6.2 log CFU/mL to 7.1 log CFU/mL, and decreased to 4.3-5.2 log CFU/mL after 5 h incubation in artificial gastric fluid. Viability was 60.6-81.9%. Simultaneously, the final number of bacteria in MRS broth was 6.3-7.1 log CFU/mL, and viability ranged from 98.8% to 104.2%.

Viability in artificial duodenal fluid. All isolates survived better in MRS broth than in artificial duodenal fluid (Table 4). The population of *Lb. acidophilus* was reduced in artificial duodenal fluid from initial 6.2-7.0 log CFU/mL to 2.4-5.8 log CFU/mL, and viability was 35.1-87.3%. These results pointed that viability of *Lb. acidophilus* depended on the isolates. For example, isolates No 4, 8, 9, and 10 survived better than isolates No 5 and 7 did. At the same time of incubation the viability in MRS broth ranged from 101.0% to 103.0%.

Table 4. The viability of *Lb. acidophilus* 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	6.8 ±0.3	3.8 ±0.4	3.0 ±0.2	56.1 ±4.2	6.9 ±0.4	–0.1 ±0.1	101.8 ±1.2
2	6.8 ±0.5	3.8 ±0.6	3.0 ±0.3	55.3 ±5.6	7.1 ±0.4	–0.1 ±0.1	101.4 ±1.0
3	6.8 ±0.5	3.6 ±0.8	3.2 ±0.5	53.1 ±9.2	6.8 ±0.2	–0.1 ±0.2	101.0 ±3.1
4	6.6 ±0.4	5.8 ±0.4	0.8 ±0.2	87.3 ±2.3	6.6 ±0.2	–0.2 ±0.1	102.5 ±1.3
5	6.5 ±0.5	2.4 ±0.6	4.1 ±0.5	36.1 ±7.5	7.1 ±0.6	–0.2 ±0.1	103.0 ±1.8
6	6.6 ±0.2	3.8 ±0.5	2.8 ±0.6	57.7 ±8.8	6.8 ±0.3	–0.1 ±0.2	101.5 ±2.4
7	7.0 ±0.1	2.4 ±0.4	4.5 ±0.5	35.1 ±6.1	7.1 ±0.1	–0.1 ±0.1	101.5 ±1.8
8	6.5 ±0.5	5.6 ±0.1	0.9 ±0.6	86.0 ±7.6	6.9 ±0.2	–0.2 ±0.2	102.8 ±2.6
9	6.7 ±0.5	5.5 ±0.3	1.2 ±0.7	82.3 ±9.4	6.8 ±0.5	–0.1 ±0.1	101.8 ±1.5
10	6.2 ±0.1	5.3 ±0.4	0.9 ±0.4	85.0 ±6.5	6.8 ±0.2	–0.2 ±0.2	102.5 ±3.3

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

To exert beneficial effect in human organism, *Lb. acidophilus* needs to survive in the human gastrointestinal tract. The ability of bacteria to survive passage through GIT is mainly attributed to their acid (of gastric fluid) and bile (of duodenal fluid) tolerance [Taranto et al. 2003, Saito 2004]. Conway et al. [1987] showed that the ability of LAB to survive in GIT varies according to the species.

The previous research indicated that the viability of isolates of LAB (bifidobacteria, *Lb. acidophilus, Lb. delbrueckii* subsp. *bulgaricus, Lb. plantarum, Lb. rhamnosus*, *Lb. casei, Lactococcus lactis, Streptococcus thermophilus*) in simulated duodenal fluid (pH was 7.0) did not depend on the cholesterol addition (ca. 500 µg/mL) but depended on the initial count of bacteria. The highest initial number of bacteria the highest number of bacterial cells survived in simulated duodenal fluid [Ziarno 2007 a]. The result of the present study suggested that *Lb. acidophilus* strains survived in gastric or duodenal fluids could participate in the reduction of cholesterol level before its absorption by human organism. Even if the bacteria had no possibility to grow in artificial GIT fluids, the cholesterol uptake by bacteria cells took place. These results are compatible with the observations of Brashears and Gilliland [1995] as well as Liong and Shah [2005]. Liong

and Shah [2005] showed that intact cells could remove cholesterol at different temperature of incubation (between 10°C and 70°C) and after the autoclaving. The results of heat treatment on the binding activity showed that binding of cholesterol was not largely affected by autoclaving. It suggested that binding of cholesterol to the bacterial cells may be a physical phenomenon and cell wall related. Brashears and Gilliland [1995] showed the ability of three strains of *Lb. acidophilus* to uptake of cholesterol during refrigerated storage. It meant that binding of cholesterol to the bacterial cells not depended on the viability of bacteria. The experiments of Tabuchi et al. [2004] suggested that viable cells and heat-sterilized cells might absorb the cholesterol in the fermented dairy products as well as in the small intestine due to their binding action. Hosono and Tono-Oka [1995] confirmed that binding of cholesterol with intact cells of lactic acid bacteria occurs very quickly and does not need time.

CONCLUSIONS

1. Studied *Lb. acidophilus* isolates showed various abilities to uptake of cholesterol from MRS broth and artificial GIT fluids.
2. Even if the bacteria had not possibility to grow in artificial GIT fluids, the cholesterol uptake by bacteria cells took place.
3. *Lb. acidophilus* isolates survived worse in simulated gastric or duodenal fluids than in MRS broth.

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USUWANIE CHOLESTEROLU W WARUNKACH *IN VITRO* PRZEZ IZOLATY *LACTOBACILLUS ACIDOPHILUS*

Streszczenie. Celem badań było określenie zdolności izolatów *Lb. acidophilus* do usuwania cholesterolu w warunkach modelowego soku żołądkowego i modelowego soku trzustkowego. Badane izolaty *Lb. acidophilus* wykazały się różną zdolnością do usuwania cholesterolu z bulionu MRS i modelowych soków trawiennych. Z modelowego soku trzustkowego izolaty *Lb. acidophilus* usunęły więcej cholesterolu (od 0,6% do 2,9%) niż z modelowego soku żołądkowego (od 3,8% do 13,5%). Ubytek cholesterolu był obserwowany, nawet jeśli komórki bakterii nie miały możliwości rozwoju w modelowych sokach trawiennych. Izolaty *Lb. acidophilus* przeżywały gorzej w modelowym soku żołądkowym lub modelowym soku trzustkowym niż w bulionie MRS. Początkowa liczba bakterii była

w zakresie 6,2-7,1 log jtk/cm³, a przeżywalność w modelowym soku żołądkowym wyniosła 60,6-81,9% logarytmu liczby początkowej bakterii. Przeżywalność w modelowym soku trzustkowym osiągnęła 35,1-87,3% logarytmu liczby początkowej bakterii.

Słowa kluczowe: cholesterol, *Lactobacillus acidophilus*, usuwanie cholesterolu, modelowy sok żołądkowy, modelowy sok trzustkowy

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