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SURVIVAL OF LACTIC ACID BACTERIA IN SIMULATED DUODENAL FLUID DEPENDING ON CHOLESTEROL PRESENCE

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Key words: lactic acid bacteria, gastrointestinal tract, survival, LAB, simulated duodenal fluid, cholesterol

The aim of this study was to compare the survival of chosen isolates of LAB in artificial duodenal fluid contained the cholesterol (0.5 g/L) with the survival in control duodenal fluid without the cholesterol addition. Isolates of five species of LAB (bifidobacteria, *Lactobacillus acidophilus, Lb. delbrueckii* subsp. *bulgaricus, Lb. plantarum, Lb. rhamnosus*) isolated from commercial pharmaceuticals and isolates of six species of LAB (bifidobacteria, *Lb. acidophilus, Lb. delbrueckii* subsp. *bulgaricus, Lb. casei, Lactococcus lactis, Streptococcus thermophilus*) originated from commercial dairy products or dairy starter cultures were used in the study. Duodenal fluid contained 5.0 g/L of NaCl, 0.6 g/L of KCl, 0.03 g/L of $CaCl_2$, 17 g/L of bile salts, and the enzyme complex (pancreatin enzymes, lipases, amylases, and proteases) dissolved in 1 M $NaHCO_3$. The pH of the juice was 7.0 ±0.2. The survival rate of studied LAB in simulated duodenal fluid did not depend on the cholesterol addition. The LAB Isolates originated from pharmaceuticals survived in simulated duodenal fluid as well as the isolates originated from dairy starter cultures or dairy products. The survival rate of studied LAB in simulated duodenal fluid depended on the initial count of bacteria. The highest initial number of LAB the highest number of bacterial cells survived in simulated duodenal fluid.

INTRODUCTION

Lactic acid bacteria (LAB) are beneficial to human health and thus some strains of them are probiotics. To exert the beneficial effect in human organism, LAB needs to survive in the gastrointestinal tract (GIT). The human gastrointestinal tract can be divided into 4 anatomical regions: the esophagus, the stomach, the small intestine (consisting of the duodenum, jejunum, and ileum), and the large intestine or colon. The ability of LAB to survive passage through GIT is mainly attributed to their acid (in the stomach) and bile tolerance (in the duodenum). There are many results of *in vivo* or *in vitro* studies concerning the survival of LAB in GIT [Hood & Zottola 1988; Marteau *et al.,* 1997; Elli *et al.,* 2006]. The main factors influencing the survival of LAB in GIT are: low pH in the stomach, intestinal peristalsis, bile salts in pancreatic juice and different digestive enzymes present on each sections of GIT. They cause the increase of survivability of LAB, but simultaneously determine the criterion of the selection of probiotic strains [Hoier, 1992; Jakubczyk & Kosikowska, 2000]. There are the data on the ability of LAB to survive during the transit through the sections of GIT, mainly through the small intestine. 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 [Gawęcki & Hryniewiecki, 1998; Michajlik & Ramotowski, 2003]. The pH of the 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.

The bile salts are the serious barrier for the LAB, because of the presence of bile acids toxic for bacterial cells [Kailasapathy & Chin, 2000; Bezkorovainy, 2001]. Lankaputhra & Shah [1995] observed different survival of studied strains of *Lactobacillus* and *Bifidobacterium* suspended on the medium contained from 0 to 1.5% of bile salts. They reported that among 9 strains of bifidobacteria, *B. longum, B. pseudolongum* and *B. infantis* show the best tolerance to bile. They have also pointed out that while one strain of *B. longum* is tolerant to bile, the other strain of *B. longum* does not survive well in bile. Noh & Gilliland [1993] found the damages and the lysis of the *Lb. acidophilus* cells in the medium contained the addition of 0.3% bile salts. Bezkorovainy [2001] introduced the data of study of chosen strains of *Lactobacillus* and *Bifidobacterium* cultured by 3 h in the presence of bile salts at the concentration ranged from 0 to 1.5%. He showed that bacteria survival was relative to the concentration of bile salts and the time of the exposure of the bacteria on them. Amongst examined bifidobacteria strains, *Bif. longum* 1941, *Bif. infantis* 1912 and *Bif. pseudolongum* 20099 had the greatest resistance. Amongst examined *Lb. acidophilus* strains, *Lb. acidophilus* 2404 and *Lb.*

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acidophilus 2415 characterized the best survivability. Clark & Martin [1994] have reported the best survival rates of *B. longum* in 2% and 4% of bile. Vinderola & Reinheimer [2003] found that *Lb. casei* did not deconjugate the bile salts, as to other species of the *Lactobacillus* genera did. And *Lb. casei* was resistant against bile salts. Marteau *et al.* [1997] presented the dynamic model of the stomach and small intestine, with the simulation of peristalsis. They explored the survival of same strains of *Bif. bifidum, Lb. acidophilus, Lb. delbrueckii subsp. bulgaricus,* and *Str. thermophilus* under different physiological conditions (the peristalsis, the changes in pH, the changes in concentration of the enzymes and bile salts). They found that survival of *Lb. acidophilus* and *Bif. bifidum* strains in applied dynamic model was compared with data obtained from human, *in vivo*.

There are some substances or factors having a protective activity on LAB and enhancing their survival during the passage through GIT. The foodstuffs, the proteins and fats especially, have protective effects on the bacterial cells [Ibrahim & Bezkorovainy, 1993; Drouault *et al.,* 1999]. Noh *et al.* [1997] observed that lactic acid bacteria grown well in the presence of cholesterol micelles and they were more resistant to lysis than cultures grown in their absence, due to alteration of the bacterial wall or membrane after the assimilation of the cholesterol by the cells. It suggests that cholesterol could improve the survival of LAB in GIT, especially in the duodenum.

The aim of this study was to compare the survival of chosen isolates of LAB in artificial duodenal fluid contained the cholesterol with the survival in control duodenal fluid without the cholesterol addition.

MATERIALS AND METHODS

The study involved microbiological determinations of survival of chosen isolates of LAB in artificial duodenal fluid. Isolates of five species of LAB (bifidobacteria, *Lactobacillus acidophilus, Lb. delbrueckii* subsp. *bulgaricus, Lb. plantarum, Lb. rhamnosus*) isolated from commercial pharmaceuticals and isolates of six species of LAB (bifidobacteria, *Lb. acidophilus, Lb. delbrueckii* subsp. *bulgaricus, Lb. casei, Lactococcus lactis, Streptococcus thermophilus*) originated from commercial dairy products or dairy starter cultures were used in this study. To the isolation the traditional microbiological plates methods have been applied. The following agar media and culture conditions were used: MRS Agar (Merck) for lactobacilli with anaerobic incubation (anaerocult, Merck) at 37°C for 37 h, BL (PrNP-A-86034-16) for bifidobacteria with anaerobic incubation (anaerocult, Merck) at 37°C for 37 h, M17 Agar (Merck) for lactococci with aerobic incubation at 30°C, and M17 Agar (Merck) for streptococci with aerobic incubation at 37°C. The isolates of lactobacilli and bifidobacteria have been cultured twice in MRS broth (Merck), and the isolates of lactococci and streptococci in M17 broth (Merck) at 37°C or 30°C for 24 h, then were used for study.

The artificial duodenal fluid has been prepared on the basic juice and the enzyme complex. The basic juice has been prepared according to Marteau *et al.* [1997] with some modifications. Duodenal fluid contained 5.0 g of NaCl (POCH), 0.6 g of KCl (POCH), 0.03 g of $CaCl_2$ (POCH), and 17 g of bile salts (Merck) dissolved in 1 L of 1 mol/L $NaHCO_3$ (POCH). After the autoclaving (at 121°C for 15 min) the pH of the basic juice was 7.0 ±0.2, and the enzyme complex was added (two capsule per 50 mL of juice). Pharmaceutical preparation called Kreon® 10 000 (Solvay Pharmaceuticals) 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, of chemical purity >99% (Sigma-Aldrich), was hot dissolved in 99% ethanol and Tween 80, mixed in 3:1 ratio, and then was used as cholesterol solution in experiments. The cholesterol has been added to the culture broth or the duodenal fluid to reach the final concentration 0.025 g/50 mL.

The bacterial inoculumus were 6.1-9.5 log CFU per 50 mL of the culture broth or the duodenal fluid. The LAB isolates were cultured in culture broth (MRS broth for lactobacilli and bifidobacteria or M17 broth for lactococci or streptococci) and in artificial duodenal fluid contained the cholesterol at initial concentration 0.5 g/L, as well as in the controls media without cholesterol. The experiments were performed at 37°C for 5 hours. At the beginning (immediately after the adding of the bacteria) and at the end of the experiment, the number of lactic acid bacteria was assayed using the plate method. The following agar media and culture conditions were used: MRS Agar (Merck) for lactobacilli and bifidobacteria with anaerobic incubation (anaerocult, Merck) at 37°C for 37 h, M17 Agar (Merck) for lactococci with aerobic incubation at 30°C, and M17 Agar (Merck) for streptococci with aerobic incubation at 37°C. Each LAB isolate were tested in three replicates. The survival of LAB was expressed as percentage of log of the final bacterial count (log CFU/mL) in comparison to their log of the initial count (log CFU/mL), which allows the comparison of different isolates, regardless of differences in initially counts. The survival rate was calculated in each trial, then the mean and SD have been calculated.

The survival of LAB in artificial duodenal fluid was compared with their survival in culture broth using the multifactor ANOVA. The result obtained for survival in media contained cholesterol were compared with the data of LAB survival in media without cholesterol addition. The statistical analysis of results was carried out with STATGRAPHICS Plus 5.1 programme.

RESULTS AND DISCUSSION

LAB, especially probiotic strains, are used as pharmaceuticals or starter cultures in the manufacture of food stuffs. As mentioned before, to health benefits, they must express high tolerance to acid, bile salts, and be able to bind to the epithelial cells of GIT [Greene & Klaenhammer 1994]. During the passage through the human GIT, bacterial cell membrane is the primary target for the substances present in the environment. Therefore, cell membrane changes its physical properties or fluidity in response to the environment. Taranto *et al.* [2003] observed that *Lb. reuteri* had changed its cellular structure in the presence of cholesterol, due to cholesterol assimilation by bacterial cells.

In this work, studied LAB showed ability to survive in simulated duodenal fluid depending on the species of LAB (Tables 1-5). Conway *et al.* [1987] also showed that the ability of LAB to survive in GIT varies according to the species. The studied strains included two *Lb. acidophilus* strains, *Lb. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus*. Noh *et al.* [1997] observed that LAB cells grown in the presence of cholesterol were more resistant to lysis than cells grown in its absence.

In the present work, *Lb. delbrueckii* subsp. *bulgaricus* strains survived better in artificial duodenal fluid than studied bifidobacteria and similar to *Lb. acidophilus* strains (Table 1). The survival rate ranged from 38.0%±2.2 to 82.0%±1.8, aside from the origin of the lactobacilli and the presence of cholesterol (p=0.1047 and p=0.8922, respectively). In the papers, there are conflicting results of studies concerning the survival of *Lb. delbrueckii* subsp. *bulgaricus* in GIT. Some authors reported that *Lb. delbrueckii* subsp. *bulgaricus* was not bile resistant and did not survive the passage through the intestinal tract, and was not recovered from faeces of humans after daily yoghurt ingestion [Pedrosa *et al*., 1995; Del Campo *et al*., 2005; Mater *et al*., 2005]. Elli *et al*. [2006] confirmed that yoghurt bacteria, especially *Lb. delbrueckii* subsp. *bulgaricus*, can be retrieved from faeces of healthy individuals after a few days of ingestion of commercial yoghurt. It could suggest that there are some undefined factors influencing the ability of LAB to survive in duodenal fluid. Moreover, other LAB, for example *Lb. acidophilus* and *B. bifidum,* have the ability to establish *Lb. delbrueckii* subsp. *bulgaricus* among the gut flora [Kailasapathy & Chin, 2000].

Three isolates of *Bifidobacterium* originated from pharmaceuticals showed the same ability to survive in simulated duodenal fluid as three isolates of bifidobacteria originated from commercial dairy starter culture (p=0.5650; Table 2). The mean survival of bifidobacteria in simulated duodenal fluid ranged from 0% to 50.0% ±0.7 and depended in the initial count of bacteria (p=0.0002). Statistical analysis confirmed that the presence of the cholesterol had not a statistically significant effect on this ability (p=0.4858).

The initial count of *Lb. acidophilus* isolates ranged from 4.6±0.5 log CFU/mL to 7.5±0.3 log CFU/mL, and after culturing in artificial duodenal fluid decreased to 2.3 – 5.2 log CFU/mL (Table 3), independent on the cholesterol presence (p=0.1905). The survival rate depended on the origin of bacteria (p=0.0001) and initial number of bacteria (p=0.0000). *Lb. acidophilus* isolated from dairy starter culture survived better in artificial duodenal fluid than isolates originated from pharmaceuticals.

Three studied isolates of *Lb. plantarum* originated from pharmaceuticals were found to show a tolerance to the consecutive exposure to artificial duodenal fluid. The resistance of *Lb. plantarum* strains to bile salts was observed by many

TABLE. 1. The count of *Lb. delbrueckii* subsp. *bulgaricus* isolated from pharmaceuticals (ph1, ph2, ph3) and starter cultures (sc1, sc2, sc3) in control broth or in the duodenal fluid without and with cholesterol addition (mean and standard deviation of 3 determinations).

Isolates no	Inoculum (log CFU/mL)	Control growth in broth				5h cultures with duodenal fluid			
		Count (log CFU/mL)		Survival (% of log CFU)		Count (log CFU/mL)		Survival (% of log CFU)	
		Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)
ph1	5.2±0.2	5.4±0.3	5.5±0.1	103.2±1.0	105.8±2.1	4.1±0.3	4.3±0.3	78.2±1.9	82.0±1.8
ph2	4.5±0.2	4.7±0.4	4.6±0.2	103.6±3.3	102.2±0.1	3.2±0.2	2.7±0.3	71.1±1.3	60.7±2.9
ph3	7.5±0.2	7.8±0.2	7.7±0.2	103.1±0.8	101.8±0.8	5.0±0.2	4.8±0.3	65.9±0.9	63.2±2.2
sc1	7.1±0.2	7.2±0.2	7.1±0.3	100.9±0.8	99.0±1.6	4.7±0.0	4.4±0.4	65.4±1.0	61.6±4.3
sc2	4.4±0.2	4.6±0.2	4.7±0.4	103.7±1.2	105.2±4.6	2.4±0.4	2.7±0.3	54.0±7.2	60.1±3.8
sc3	7.3±0.3	7.6±0.3	7.4±0.3	104.1±0.1	101.4±0.0	2.8±0.3	3.8±0.3	38.0±2.2	51.8±1.7

TABLE 2. The count of *Bifidobacterium* species isolated from pharmaceuticals (ph1, ph2, ph3) and starter cultures (sc1, sc2, sc3) in control broth or in the duodenal fluid without and with cholesterol addition (mean and standard deviation of 3 determinations).

Isolates no	Inoculum (log CFU/mL)	Control growth in broth				5h cultures with duodenal fluid			
		Count (log CFU/mL)		Survival (% of log CFU)		Count (log CFU/mL)		Survival (% of log CFU)	
		Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)
ph1	6.3±0.3	6.5±0.2	6.3±0.3	103.2±1.7	101.1±1.8	2.7±0.1	2.4±0.4	43.1±0.2	38.2±4.9
ph2	5.9±0.1	5.9±0.1	5.8±0.2	100.0±1.7	98.9±2.0	2.1±0.3	2.7±0.2	35.1±4.8	45.2±3.3
ph3	7.8±0.1	7.7±0.1	7.7±0.3	99.6±0.7	99.6±3.0	1.3±0.3	1.9±0.4	16.8±4.0	24.1±4.7
sc1	4.7±0.3	4.9±0.3	5.0±0.3	103.5±1.2	105.6±1.2	0.0±0.0	1.0±0.1	0.0±0.0	21.8±0.5
sc2	5.3±0.3	5.5±0.2	5.5±0.2	104.5±3.2	103.9±2.1	2.5±0.2	2.6±0.2	47.4±6.5	49.3±6.6
sc3	7.0±0.5	7.2±0.5	7.2±0.3	102.9±1.6	103.0±3.1	3.5±0.3	3.1±0.2	50.0±0.7	44.3±0.3

TABLE. 3. The count of *Lb. acidophilus* isolated from pharmaceuticals (ph1, ph2, ph3) and starter cultures (sc1, sc2, sc3) in control broth or in the duodenal fluid without and with cholesterol addition (mean and standard deviation of 3 determinations).

Isolates no	Inoculum (log CFU/mL)	Control growth in broth				5h cultures with duodenal fluid			
		Count (log CFU/mL)		Survival (% of log CFU)		Count (log CFU/mL)		Survival (% of log CFU)	
		Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)
ph1	6.5±0.4	6.6±0.1	6.7±0.2	101.7±4.7	102.7±4.0	4.6±0.2	4.8±0.3	70.3±2.0	73.8±0.1
ph2	5.9±0.7	6.2±0.2	6.0±0.2	106.3±8.5	103.5±9.2	4.7±0.2	4.8±0.2	80.5±5.6	81.7±6.5
ph3	4.6±0.5	5.3±0.3	5.1±0.2	116.4±5.1	112.9±8.2	3.3±0.3	3.8±0.5	72.3±0.8	82.4±1.8
sc1	5.0±0.2	5.2±0.2	5.1±0.1	103.3±1.1	102.0±2.0	2.3±0.3	2.5±0.2	46.3±3.6	50.3±1.5
sc2	7.5±0.3	7.6±0.2	7.7±0.1	101.9±6.1	102.8±5.5	4.7±0.3	5.2±0.3	63.3±5.9	69.9±6.2
sc3	6.5±0.3	6.8±0.2	6.7±0.2	104.2±2.5	102.6±2.4	3.3±0.2	3.8±0.3	51.3±0.5	57.9±1.3

TABLE 4. The count of *Lb. plantarum* and *Lb. rhamnosus* isolated from pharmaceuticals in control broth or in the duodenal fluid without and with cholesterol addition (mean and standard deviation of 3 determinations).

Isolates no	Inoculum (log CFU/mL)	Control growth in broth				5h cultures with duodenal fluid			
		Count (log CFU/mL)		Survival (% of log CFU)		Count (log CFU/mL)		Survival (% of log CFU)	
		Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)
Lb. plantarum									
ph1	7.5±0.2	7.7±0.2	7.6±0.3	102.7±0.1	100.9±0.8	6.2±0.2	6.2±0.2	82.2±0.4	82.7±0.5
ph2	5.9±0.2	6.2±0.2	6.0±0.2	105.1±0.2	101.7±0.1	4.8±0.3	4.9±0.4	80.8±1.6	82.4±3.2
ph3	7.8±0.2	8.0±0.2	7.9±0.3	102.6±0.1	101.3±1.3	6.3±0.3	5.8±0.1	80.7±1.7	74.7±0.3
Lb. rhamnosus									
ph1	7.7±0.3	7.8±0.1	7.8±0.3	101.8±2.1	101.3±0.0	6.7±0.1	6.7±0.3	87.4±1.6	86.9±0.4
ph2	5.5±0.3	5.6±0.3	5.6±0.5	101.2±1.1	101.7±4.9	4.5±0.1	4.5±0.3	81.9±2.7	81.8±1.0
ph3	7.3±0.3	7.5±0.2	7.4±0.3	103.2±0.9	101.4±0.0	6.6±0.3	6.8±0.3	90.4±0.3	93.1±0.2

scientists both for strains isolated from intestinal samples and for those isolated from fermented foods [Haller *et al.,* 2001; De Vries *et al.,* 2006]. This work confirmed the ability of *Lb. plantarum* isolates to survive in artificial duodenal fluid. The mean survival rate was 80.6% (Table 4) and did not depend on cholesterol presence (p=0.0607). The number of survived bacterial cells did not depend on the initial count of bacteria (p=0.0009). The lowest initial number of lactobacilli the lowest number of survived bacterial cells. Strain of *Lb. plantarum* studied *in vivo* by Vesa *et al.* [2000] also displayed high survival in human GIT. Survival was tested after a single dose of 10^8 cells was given to healthy volunteers. Survival of those *Lb. plantarum* up to 25% was reached in the faeces after 1 week of daily ingestion. However, Johansson *et al.* [1993] demonstrated that survival of *Lb. plantarum* could be individual-dependent. Johansson *et al.* [1993] showed also that survival of *Lb. plantarum* was better that of other lactobacilli (*Lb. reuteri, Lb. acidophilus,* and *Lb. casei*).

Experiments confirmed the ability of *Lb. rhamnosus* isolates originated from pharmaceuticals to survive in simulation of human duodenal fluid (Table 4), apart from cholesterol presence (p=0.9122). The mean survival rate was 86.9% (Table 4) and depended on initial count of studied bacteria (p=0.0002). The highest initial number of *Lb. rhamnosus* the highest number of bacterial cells survived in simulated duodenal fluid.

Three cultures of *Lb. casei* isolated from dairy product showed the ability to survive in simulated duodenal fluid at level 73.4% (Table 5). The presence of the cholesterol had not a statistically significant effect on the final count of bacteria nor in simulated duodenal fluid nor in MRS broth (p=0.3158). The number of survived lactobacilli significantly depended on the initial number of tested LAB (p=0.0001). The highest initial number of *Lb. casei*, the highest number of bacterial cells survived in simulated duodenal fluid. Oozeer *et al.* [2006] concluded that *Lb. casei* could survive well during passage through GIT. The survival of *Lb. casei* studied by Oozeer *et al.* [2006] was approximately 28.4% in the faeces.

There have been few studies on the probiotic properties of bacteria from *Lactococcus* species, since they are formally not considered to be natural inhabitants of GIT. Kimoto *et al.* [2003] investigated ability of lactococci to survive during mouse GIT by *in vitro* and *in vivo* tests. Viable cells of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* N7 were

TABLE 5. The count of *Lb. casei, Lactococcus lactis* subsp. *lactis* and *Streptococcus thermophilus* isolated from dairy starter cultures in control broth or in the duodenal fluid without and with cholesterol addition (mean and standard deviation of 3 determinations).

Isolates no	Inoculum (log CFU/mL)	Control growth in broth				5h cultures with duodenal fluid			
		Count (log CFU/mL)		Survival (% of log CFU)		Count (log CFU/mL)		Survival (% of log CFU)	
		Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)	Without cholesterol	With cholesterol (0.5 g/L)
Lb. casei									
sc1	5.8±0.2	6.0±0.2	6.1±0.3	103.5±0.1	104.6±0.9	4.2±0.2	4.2±0.2	72.4±1.0	72.4±1.0
sc2	6.7±0.3	6.8±0.2	6.7±0.3	102.0±1.0	100.0±0.0	4.8±0.3	4.8±0.3	71.5±1.1	71.5±1.1
sc3	7.2±0.3	7.3±0.2	7.2±0.3	101.4±1.4	99.6±0.8	5.5±0.3	5.5±0.1	76.4±1.0	76.4±1.8
Lactococcus lactis subsp. *lactis*									
sc1	5.8±0.3	5.7±0.2	5.8±0.3	98.9±1.0	100.0±0.0	3.4±0.1	3.5±0.3	59.0±0.9	60.1±1.8
sc2	4.9±0.4	4.8±0.3	4.9±0.3	97.4±2.9	99.5±3.0	2.2±0.2	2.1±0.2	44.9±0.4	43.6±0.8
sc3	6.4±0.4	6.5±0.2	6.5±0.2	101.7±3.2	101.7±3.2	4.6±0.1	4.5±0.3	72.0±2.9	70.3±0.3
Streptococcus thermophilus									
sc1	5.3±0.4	5.4±0.3	5.4±0.1	100.7±2.1	101.5±4.8	1.9±0.3	2.0±0.1	34.9±2.6	37.5±0.6
sc2	7.0±0.3	7.1±0.3	7.0±0.2	101.0±0.8	100.0±1.4	4.6±0.2	4.7±0.1	65.7±0.0	67.2±1.5
sc3	5.9±0.2	5.8±0.3	5.9±0.2	97.2±2.0	99.4±1.0	2.0±0.2	2.1±0.1	33.7±2.5	35.4±0.8

recovered from faeces within 24-48 h after administration to mice but not at 72 h. Other strain, *L. lactis* subsp. *cremoris* ATCC 19257, which had a poor survival rate *in vitro* test, was also detected at 12 h but not at 24 h. Vesa *et al.* [2000] demonstrated that *L. lactis* survived only at level 1% ±0.8 in the duodenum. *L. lactis* subsp. *lactis* studied in this work showed good resistance to artificial duodenal fluid and the mean survival was 58.3% (Table 5). The presence of the cholesterol had not a statistically significant effect on this ability (p=0.8899), but the initial number of bacteria did (p=0.0001).

Other paper suggested that food may have a important protective effect on the bacteria present in diet. Uptake of pure *L. lactis* culture led to a massive cell lysis in the digestive tract, but *L. lactis* mixed with food survived well in the duodenum [Drouault *et al.,* 1999].

Strains of *L. lactis* subsp. *lactis*, *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are used for production of traditional milk products: cheeses, sour cream, and fermented milk. The concentration of these organisms in the human or animal gastrointestinal tract has been poorly examined in comparison with that of other probiotic strains. Some authors reported that non-probiotic LAB does not survive during the passage and does not colonize in GIT.

There have been conflicting studies concerning the survival of *S. thermophilus* in GIT after daily yoghurt ingestion [Pedrosa *et al.,* 1995; Del Campo *et al.,* 2005; Elli *et al.,* 2006]. Some authors reported that *S. thermophilus* were not recovered from faeces of subjects [Pedrosa *et al.,* 1995; Del Campo *et al.,* 2005]. Brigidi *et al.* [2003] recovered *S. thermophilus* from faecal samples for 6 days after the end of intake of a pharmaceutical preparation orally for 3 days. The persistence of a yoghurt culture in the human gut was also confirmed by Mater *et al.* [2005], who studied 13 healthy volunteers fed yoghurt containing strains of *S. thermophilus*. In study carried out by Elli *et al.* [2006], *S. thermophilus* was retrieved from only one volunteer on day 7. *S. thermophilus* is not bile resistant and does not survive the passage through the intestinal tract. However, *Lb. acidophilus* and bifidobacteria have the ability to establish it among the gut flora [Kailasapathy & Chin, 2000].

In this paper, *S. thermophilus* survived in simulated duodenal fluid as well as bifidobacteria isolates did (Table 5). The mean survival rate was 45.7% and depended on the initial count of bacteria (p=0.0001) but not on the presence of cholesterol (p=0.5593). This result confirmed that yoghurt streptococci could not survive in sufficient numbers without the protective food envelope and affect on human health.

There are no data in the literature in relation the influence of the cholesterol on survival of LAB in GIT. The previous research demonstrated that yoghurt bacteria *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were able to remove cholesterol from duodenal fluid, simulated gastro-intestinal track [Ziarno & Bartosz, 2007]. The degree of the cholesterol level reduction depended on the biomass concentration. The highest average uptake of cholesterol during 5 h culture in duodenal fluid was obtained for 10-fold concentrated biomasses of *Lb. delbrueckii* subsp. *bulgaricus* (0.129 g/L ±0.044) or *S. thermophilus* (0.139 g/L ±0.029). The survival of yoghurt bacteria in the artificial duodenal fluid during 5 h grow was also investigated. *Lb. delbrueckii* subsp. *bulgaricus* survived better than *S. thermophilus*. The addition of the cholesterol did not influence on the survival rate of yoghurt bacteria.

From the survival data, it seems feasible to obtain elevated levels of viable LAB in human intestine by careful selection of the bacterial strains ingested [Conway *et al.,* 1987]. Furthermore, the *in vitro* method used here could be valuable to screen potential probiotic properties of LAB. The data obtained in this work could be correlated with human *in vivo* studies monitoring the beneficial effect of ingestion of LAB. Dunne *et al.* [2001] showed that *in vitro* study of LAB can

result in the isolation of strains capable of performing effectively in the gastrointestinal tract. This work confirmed, that the highest initial number of LAB the highest number of bacteria survived in simulated duodenal fluid.

CONCLUSIONS

1. The survival rate of studied LAB in simulated duodenal fluid does not depend on the cholesterol addition.

2. The LAB Isolates originated from pharmaceuticals survive in simulated duodenal fluid as well as the isolates originated from dairy starter cultures or dairy products.

3. The survival rate of studied LAB in simulated duodenal fluid depends on the initial count of bacteria. The highest initial number of LAB the highest number of bacterial cells survived in simulated duodenal fluid.

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WPŁYW CHOLESTEROLU NA PRZEŻYWALNOŚĆ BAKTERII MLEKOWYCH W SYMULACJI SOKU DWUNASTNICY

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Celem pracy było porównanie przeżywalności wybranych izolatów bakterii mlekowych (LAB) w modelowym soku z dwunastnicy zawierającym cholesterol (0,5 g/l) i określenie wpływu cholesterolu na tę przeżywalność. Do badań użyto izolatów pięciu gatunków LAB (bifidobakterii, *Lactobacillus acidophilus, Lb. delbrueckii* subsp. *bulgaricus, Lb. plantarum, Lb. rhamnosus*) wyizolowanych w preparatów farmaceutycznych oraz izolatów sześciu gatunków LAB (bifidobakterii, *Lb. acidophilus, Lb. delbrueckii* subsp. *bulgaricus, Lb. casei, Lactococcus lactis, Streptococcus thermophilus*) pochodzących w mleczarskich kultur starterowych i produktów mleczarskich. Modelowy sok z dwunastnicy zawierał 5,0 g/l NaCl, 0,6 g/l KCl, 0,03 g/l $CaCl_2$, 17 g/l żółci oraz zestaw enzymów (pankreatynowych, lipaz, amylaz i proteaz) rozpuszczonych w 1 M $NaHCO_3$. Wartość pH soku wynosiła 7,0 ±0,2. Wykazano, że stopień przeżywalności wszystkich badanych LAB w modelowym soku z dwunastnicy nie zależał od obecności cholesterolu. Izolaty LAB pochodzące z preparatów farmaceutycznych przeżywały w takim samym stopniu jak izolaty pobrane w mleczarskich kultur starterowych lub produktów mleczarskich. Stopień przeżycia badanych LAB w modelowym soku z dwunastnicy zależał od początkowej liczby bakterii. Im wyższa była początkowa liczba wprowadzonych bakterii, tym więcej ich przeżywało w modelowym soku z dwunastnicy.