ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Technol. Aliment. 9(1) 2010, 83-94*
ISSN 1644-0730 (print) ISSN 1889-9594 (online)

# VIABILITY AND CHOLESTEROL UPTAKE BY *STREPTOCOCCUS THERMOPHILUS* CULTURES IN ARTIFICIAL GIT FLUIDS*

Małgorzata Ziarno
Warsaw University of Life Sciences – SGGW

**Background.** *Streptococcus thermophilus* is traditionally used in association with *Lb. delbrueckii* subsp. *bulgaricus* as a starter culture for the production of yoghurt. Some researchers have indicated that *S. thermophilus* may provide additional health benefits, for example it may reduce cholesterol levels. The aim of this study was to *in vitro* evaluate the cholesterol uptake and the viability of *S. thermophilus* isolates in artificial GIT environments.
**Material and methods.** Twelve isolates of *S. thermophilus* were cultured in artificial gastric fluid (with pepsin added) and in artificial duodenal fluid (with the enzyme complex added), and in M17 broth containing cholesterol at an initial concentration of 600 µg/mL, as well as in M17 broth without cholesterol. Immediately after the adding of bacteria inoculums and at the end of experiment, the concentration of cholesterol and the number of bacteria were measured.
**Results.** *S. thermophilus* did not remove statistically significant amounts of cholesterol from artificial gastric fluid. The isolates showed the ability to uptake cholesterol from M17 broth and artificial duodenal fluid, and the degree of cholesterol uptake depended on the isolate. All isolates of *S. thermophilus* remove much more cholesterol from M17 broth than from artificial duodenal fluid. All *S. thermophilus* isolates had worse survival in artificial gastric or duodenal fluids than in M17 broth.
**Conclusions.** The ability of *S. thermophilus* cells to survive in artificial gastric fluid and artificial duodenal fluid varied according to the isolates.

**Key words:** cholesterol, *Streptococcus thermophilus*, cholesterol uptake, artificial intestinal juice, artificial duodenal juice

*This paper is supported by the grant from Warsaw University of Life Sciences – WULS--SGGW.



Corresponding author – Adres do korespondencji: Dr hab. 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

*Streptococcus thermophilus* belongs to the homofermentative thermophilic group of the lactic acid bacteria (LAB). It is traditionally used in association with *Lb. delbrueckii* subsp. *bulgaricus* as a starter culture for the production of yoghurt. A major metabolite of *S. thermophilus* and a contributor to the yoghurt flavour is acetaldehyde. *S. thermophilus* is also paired with other thermophilic *Lactobacillus* species for the production of Swiss and Italian cheeses (Emmental and Mozzarella). It has also been used with lactococci in the "short method" of Cheddar production [Michel and Martley 2001, Gagnaire et al. 2004].

*S. thermophilus* is highly adapted to the dairy environment and is isolated at low levels from raw milk. It ferments a limited number of sugars including lactose, fructose, sucrose and glucose. It requires free amino acids for growth, including glutamic acid, histidine, methionine, cysteine, valine, leucine, iso-leucine, tryptophan, arginine and tyrosine. These amino acids are provided by the proteolytic *Lactobacillus* sp. and the *Lb. delbrueckii* subsp. *bulgaricus*, for example. Some strains of *S. thermophilus* produce exopolysaccharides (EPS). These "ropey" strains are often used for products such as yoghurt [Hassan et al. 2003, Shene et al. 2008].

Some researchers have indicated that *Streptococcus thermophilus* may provide additional health benefits [Thibault et al. 2004, Heyman et al. 2005, Menard et al. 2005]. There is no evidence that *S. thermophilus* has any significant ability to act as an opportunistic pathogen. It is interesting to note that while it has been claimed that yoghurt and various *Lactobacillus* strains have a probiotic activity, no probiotic claims have been made to date for *S. thermophilus*. Bacteriocin activity against closely related bacteria has been described in *S. thermophilus* supernatants. Some strains also exhibit inhibitory activity against bacteria species such as *Bacillus*, *Staphylococcus*, *Listeria*, *Salmonella*, *Escherichia coli* and *Yersinia species* [Ivanova et al. 1998, Benkerroum et al. 2002, Medici et al. 2005].

Some *in vitro* and *in vivo* research indicates that strains of *S. thermophilus* may reduce cholesterol levels [Agerholm-Larsen et al. 2000, Dilmi-Bouras 2006, Ziarno et al. 2007], but in general the researchers demonstrated that yoghurt bacteria consumption had varied effects on cholesterol in blood serum [Akalin et al. 1997, Agerholm-Larsen et al. 2000, Kiessling et al. 2002]. For example, Akalin et al. [1997] showed that the serum cholesterol concentrations and LDL cholesterol concentrations were significantly decreased when the mice were fed acidophilus yoghurt compared to standard yoghurt. Additionally, neither HDL cholesterol levels nor triglyceride levels were affected by yoghurt. Agerholm-Larsen et al. [2000] proved that hypocholesterol activity of *S. thermophilus* was enhanced by addition of probiotic *Lactobacillus* strains. Kiessling et al. [2002] demonstrated that long-term daily consumption of yoghurt over a period of 21 weeks increased the serum concentration of HDL cholesterol and lead to the desired improvement of the LDL/HDL cholesterol ratio. The results obtained by Dilmi-Bouras [2006] showed that certain strains of *S. thermophilus* resist bile salts and assimilate appreciable quantities of cholesterol quantities. However, the symbiotic effect between *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*, with regard to bile salts, was observed.

The mechanisms of hypocholesterol activity of LAB are not completely understood. They are explained by many potential hypotheses, including physiological actions of the

end products of short-chain fatty acid (SCFA), cholesterol assimilation by the bacterial cells, cholesterol binding to the bacterial cell wall, and enzymatic deconjugation of bile salts [Hosono and Tono-Oka 1995, Kimoto et al. 2002, Pereira and Gibson 2002]. These hypotheses need to be confirmed by *in vitro* and *in vivo* studies, as well as in the human gastrointestinal track (GIT) environment, where hypocholesterol activity may be influenced by numerous factors, such as anaerobic conditions, presence of bile salts, viability and number of bacterial cells [Bezkorovainy 2001, Pereira and Gibson 2002, Lim et al. 2004]. Additionally, most researchers investigate probiotic strains of the genera *Lactobacillus* and *Bifidobacterium*, and typical starter bacteria such as *S. thermophilus* have only rarely been used in animal or human *in vivo* studies, because they are not considered to exert health benefits comparable to those of probiotic strains.

The aim of this study was to evaluate the *in vitro* viability and cholesterol uptake of *Streptococcus thermophilus* isolates in artificial GIT environments in comparison to isolates in M17 broth.

## MATERIAL AND METHODS

**Sources and maintenance of cultures.** Twelve isolates of *Streptococcus thermophilus*, which originated from commercial yoghurts (containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*), were used in this study. The traditional microbiological plate method (M17 Agar, Merck, Germany, at 37°C/72 h under aerobic conditions) has been applied to the isolation procedure. The bacteria were cultured twice in M17 broth (Merck, Germany) at 37°C/14 h, and then were used for experiments.

**Artificial gastric fluid.** Artificial gastric fluid was prepared by supplementing basic gastric fluid with pepsin. The basic gastric fluid was 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 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) was added per 50 mL of the artificial gastric fluid.

**Artificial duodenal fluid.** Artificial duodenal fluid was prepared by supplementing the basic duodenal fluid with the enzyme complex. The basic duodenal fluid was 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 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 capsules per 50 mL of fluid). The pharmaceutical preparation called Kreon® 10 000 (Solvay Pharmaceuticals, USA) was used as the source of the enzyme complex. One capsule of Kreon® 10 000 contains 150 mg of pancreatic enzymes: 10,000 F.I.P. units of lipases, 8000 F.I.P. units of amylases, and 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 a 3:1 ratio, and then was used as the cholesterol solution in experiments. Cholesterol was added to the culture broth or artificial GIT fluids to reach the final concentration of 600 µg/mL.

**The experiments.** *Streptococcus thermophilus* isolates were cultured in M17 broth and in artificial GIT fluids containing cholesterol at an initial concentration of 600 µg/mL, as well as in M17 broth without cholesterol. The experiments were performed at 37°C for 5 h (in artificial gastric fluid and M17 broth) or at 37°C/6 h (in artificial duodenal fluid and M17 broth). Immediately after the adding of bacteria inoculums and at the end of experiment, the concentration of cholesterol and the number of bacteria were measured. The initial bacterial inoculums were 6.7-7.2 log CFU/mL in M17 broth and artificial GIT fluids.

**Measurement of number of bacteria.** The number of bacteria was assayed using the plate method. M17 Agar (Merck, Germany) with aerobic incubation at 37°C/72 h was used for quantitation of *Streptococcus thermophilus*. Each isolate was tested in five independent experiments. The viability of bacteria was expressed as the percentage of the log of the initial bacterial count (log CFU/mL) in comparison to the log of the final count (log CFU/mL). The viability was calculated in each experiment, as were the mean and the SD.

**Measurement of cholesterol uptake.** Cholesterol uptake was assayed by using 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 Helios Gamma spectrophotometer (Thermo Electron Corporation, USA) at 500 nm. Prior to assaying the cholesterol concentration in the broth, the tubes were centrifuged (6000 rpm for 7 min at 4°C) to remove the bacterial cells and biomass and obtain a clear broth supernatant. The bacterial isolates' ability to uptake cholesterol was calculated as a decrease in cholesterol concentration in broth supernatant after the end of the experiment. The percent of cholesterol removed from the broth during the growth of bacteria has been expressed as the percent of the initial concentration of cholesterol.

**Statistical analysis.** Cholesterol uptake of *Streptococcus thermophilus* isolates in artificial GIT fluids was compared with cholesterol uptake from M17 broth using multifactor ANOVA (at the 95.0% confidence level). The statistical analysis of results was carried out using the Statgraphics Plus 5.1 software.

## RESULTS

**Cholesterol uptake in GIT fluids.** The isolates of *S. thermophilus* used in this study demonstrated varied ability to uptake cholesterol from M17 broth, artificial gastric fluid and duodenal fluid. The results are presented in Tables 1 and 2.

**Cholesterol uptake in artificial gastric fluid.** The initial cholesterol concentrations in the *S. thermophilus* cultures ranged from 587 µg/mL for isolates N°12 to 630 µg/mL for isolates N°7 (Table 1). The cholesterol uptake depended significantly on the culture environment (p-value = 0.0399), and strain (p-value = 0.0105). In artificial gastric fluid, none of the isolates removed significant amounts of cholesterol (p-value = 0.2519). All isolates significantly reduced the cholesterol level during the 5 h incubation in M17 broth (p-value = 0.0001). Isolate N°8 removed the lowest amount of cholesterol from M17 broth (av. 13 µg/mL ±3), while isolate N°3 removed 38 µg/mL of cholesterol from M17 broth. The percentage of cholesterol removed from M17 broth ranged from 2.2--6.3%.

Table 1. The uptake of cholesterol by *Streptococcus thermophilus* isolates in artificial gastric fluid and in M17 broth

| Isolate number | Initial concentration of cholesterol µg/mL | Artificial gastric fluid | | | M17 broth | | |
|---|---|---|---|---|---|---|---|
| | | concentration of cholesterol µg/mL | | percentage of cholesterol removed | concentration of cholesterol µg/mL | | percentage of cholesterol removed |
| | | after 5 h | difference | | after 5 h | difference | |
| 1 | 588 ±29 | 581 ±26 | 7 ±3 ab | 1.2 ±0.5 a | 555 ±33 | 33 ±8 ab | 5.7 ±1.5 ab |
| 2 | 602 ±34 | 594 ±34 | 8 ±3 b | 1.3 ±0.5 a | 578 ±37 | 24 ±4 c | 4.0 ±0.9 c |
| 3 | 601 ±26 | 595 ±26 | 6 ±3 abc | 1.0 ±0.5 ab | 563 ±26 | 38 ±4 b | 6.3 ±0.7 b |
| 4 | 610 ±35 | 602 ±35 | 8 ±2 b | 1.3 ±0.4 a | 589 ±35 | 21 ±4 c | 3.4 ±0.7 cd |
| 5 | 603 ±32 | 595 ±30 | 8 ±3 b | 1.3 ±0.5 a | 580 ±31 | 22 ±4 c | 3.7 ±0.7 c |
| 6 | 604 ±37 | 600 ±37 | 4 ±1 cd | 0.6 ±0.2 bc | 573 ±39 | 31 ±4 a | 5.2 ±0.9 a |
| 7 | 630 ±29 | 626 ±31 | 4 ±4 abcd | 0.7 ±0.6 bc | 595 ±28 | 35 ±7 ab | 5.5 ±1.0 ab |
| 8 | 602 ±18 | 591 ±19 | 11 ±2 e | 1.9 ±0.4 d | 589 ±17 | 13 ±3 a | 2.2 ±0.5 a |
| 9 | 598 ±25 | 590 ±24 | 8 ±2 b | 1.3 ±0.3 a | 584 ±26 | 14 ±2 ad | 2.4 ±0.4 ad |
| 10 | 605 ±24 | 597 ±25 | 8 ±2 be | 1.4 ±0.3 ad | 582 ±23 | 23 ±3 c | 3.7 ±0.5 c |
| 11 | 662 ±29 | 595 ±28 | 2 ±1 d | 0.3 ±0.1 c | 588 ±29 | 14 ±6 a | 2.3 ±0.9 e |
| 12 | 587 ±43 | 583 ±43 | 3 ±1 cd | 0.6 ±0.1 bc | 567 ±42 | 20 ±2 cd | 3.4 ±0.4 cd |

Results are expressed as mean ±standard deviation, n = 5.
Different letters in the same column indicate significant differences between means with Tuckey test ($p < 0.05$).

Table 2. The uptake of cholesterol by *Streptococcus thermophilus* isolates in artificial duodenal fluid and in M17 broth

| Isolate number | Initial concentration of cholesterol µg/mL | Artificial duodenal fluid | | | M17 broth | | |
|---|---|---|---|---|---|---|---|
| | | concentration of cholesterol µg/mL | | percentage of cholesterol removed | concentration of cholesterol µg/mL | | percentage of cholesterol removed |
| | | after 6 h | difference | | after 6 h | difference | |
| 1 | 606 ±13 | 583 ±13 | 23 ±3 ab | 3.8 ±0.5 abc | 582 ±13 | 31 ±7 ab | 5.1 ±1.1 a |
| 2 | 603 ±19 | 588 ±20 | 15 ±3 cd | 2.4 ±0.5 de | 589 ±16 | 22 ±3 cd | 3.7 ±0.5 bc |
| 3 | 609 ±37 | 582 ±32 | 27 ±6 b | 4.4 ±0.8 e | 579 ±34 | 41 ±5 e | 6.6 ±1.1 d |
| 4 | 607 ±23 | 588 ±23 | 18 ±3 ac | 3.0 ±0.5 ae | 588 ±23 | 22 ±4 cd | 3.7 ±0.8 bc |
| 5 | 580 ±20 | 560 ±18 | 20 ±4 a | 3.5 ±0.7 ab | 564 ±27 | 23 ±4 cd | 3.9 ±0.8 bc |
| 6 | 601 ±14 | 578 ±19 | 23 ±6 ab | 3.8 ±1.0 bc | 581 ±7 | 27 ±5 abd | 4.5 ±0.9 ac |
| 7 | 619 ±33 | 592 ±29 | 27 ±5 b | 4.3 ±0.6 c | 589 ±29 | 32 ±2 b | 5.2 ±0.3 a |
| 8 | 604 ±13 | 593 ±13 | 11 ±2 d | 1.9 ±0.3 d | 595 ±10 | 16 ±3 fg | 2.6 ±0.5 ef |
| 9 | 606 ±20 | 595 ±17 | 12 ±4 d | 1.9 ±0.7 d | 596 ±21 | 20 ±4 cg | 3.2 ±0.8 bf |
| 10 | 623 ±31 | 609 ±29 | 15 ±2 cd | 2.4 ±0.3 de | 611 ±10 | 24 ±6 cd | 3.8 ±1.0 bc |
| 11 | 623 ±23 | 617 ±24 | 6 ±2 e | 1.0 ±0.3 f | 622 ±14 | 10 ±3 f | 1.5 ±0.5 e |
| 12 | 605 ±19 | 582 ±21 | 23 ±3 ab | 3.8 ±0.6 bc | 584 ±15 | 26 ±7 acd | 4.2 ±1.2 abc |

Results are expressed as mean ±standard deviation, n = 5.
Different letters in the same column indicate significant differences between means with Tuckey test ($p < 0.05$).

**Cholesterol uptake in artificial duodenal fluid.** The cholesterol uptake depended significantly on the culture environment (p-value = 0.0001), and strain (p-value = 0.0001). *S. thermophilus* isolates exhibited variation with regard to cholesterol uptake in artificial duodenal fluid (Table 2). The cholesterol uptake values ranged from 6 µg/mL for isolate N°11 (1.0% of initial cholesterol level) to 27 µg/mL for isolates N°3 and 7 (4.3-4.4% of initial cholesterol level). All *S. thermophilus* isolates removed much more cholesterol from M17 broth than from artificial duodenal fluid. For the same incubation period in M17 broth, the amounts of removed cholesterol ranged from 10 µg/mL for isolate N°11, to 41 µg/mL for isolate N°3.

**Viability in artificial gastric fluid.** All of the studied *S. thermophilus* isolates had significantly worse survival in artificial gastric fluid than in M17 broth (p-value = 0.0001; Table 3). The viability of *S. thermophilus* cells in artificial gastric fluid depended on the isolate (p-value = 0.0001). The initial number of bacteria ranged from 6.8 log CFU/mL to 7.2 log CFU/mL and decreased to 3.2-5.4 log CFU/mL after a 5 h incubation in artificial gastric fluid. Viability ranged from 45.9-77.1% of the initial bacteria tier. Simultaneously, the final number of bacteria in M17 broth ranged from 6.8 log CFU/mL to 7.1 log CFU/mL (viability was 95.8-100.8%).

Table 3. The viability of *Streptococcus thermophilus* isolates in artificial gastric fluid and in M17 broth

| Isolate number | Initial number of bacteria log CFU/mL | Artificial gastric fluid | | | M17 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 | 7.1 ±0.2 | 3.9 ±0.3 | 3.2 ±0.3 a | 55.0 ±4.0 ab | 7.0 ±0.3 | 0.1 ±0.2 ab | 98.6 ±2.3 abc |
| 2 | 7.0 ±0.2 | 3.9 ±0.4 | 3.1 ±0.3 a | 56.1 ±5.4 a | 6.9 ±0.3 | 0.1 ±0.2 ab | 98.3 ±2.3 abc |
| 3 | 7.0 ±0.3 | 3.2 ±0.5 | 3.8 ±0.5 b | 45.9 ±6.6 c | 7.0 ±0.2 | 0.0 ±0.1 a | 99.5 ±1.6 c |
| 4 | 7.2 ±0.5 | 3.6 ±0.6 | 3.5 ±0.3 b | 50.3 ±6.0 bc | 6.9 ±0.5 | 0.3 ±0.1 b | 95.8 ±1.2 a |
| 5 | 6.8 ±0.1 | 4.5 ±0.2 | 2.4 ±0.2 cd | 65.2 ±2.6 de | 6.9 ±0.1 | 0.0 ±0.1 a | 100.3 ±1.9c |
| 6 | 7.1 ±0.4 | 4.1 ±0.3 | 3.0 ±0.2 a | 57.3 ±1.6 a | 6.8 ±0.1 | 0.3 ±0.4 b | 96.3 ±5.4 ab |
| 7 | 7.0 ±0.4 | 4.6 ±0.4 | 2.4 ±0.2 c | 66.2 ±3.3 de | 7.0 ±0.3 | 0.0 ±0.1 a | 100.6 ±1.3 c |
| 8 | 7.2 ±0.4 | 4.5 ±0.6 | 2.6 ±0.2 c | 62.9 ±5.1 d | 7.1 ±0.3 | 0.1 ±0.2 ab | 98.9 ±3.2 bc |
| 9 | 7.1 ±0.3 | 5.0 ±0.5 | 2.1 ±0.2 d | 70.0 ±4.1 ef | 7.1 ±0.3 | 0.0 ±0.1 a | 100.6 ±1.6 c |
| 10 | 7.1 ±0.3 | 5.4 ±0.4 | 1.8 ±0.2 e | 75.3 ±2.8 fg | 7.1 ±0.3 | 0.1 ±0.1 ab | 98.9 ±1.2 bc |
| 11 | 7.0 ±0.2 | 5.4 ±0.3 | 1.6 ±0.2 e | 77.1 ±2.9 g | 7.0 ±0.2 | 0.0 ±0.2 a | 99.5 ±2.4 c |
| 12 | 7.0 ±0.2 | 4.8 ±0.3 | 2.2 ±0.2 d | 68.8 ±3.0 e | 7.1 ±0.3 | –0.1 ±0.1 a | 100.8 ±0.8 c |

Results are expressed as mean ±standard deviation, n = 5.
Different letters in the same column indicate significant differences between means with Tuckey test ($p < 0.05$).

**Viability in artificial duodenal fluid.** The viability of *S. thermophilus* isolates in artificial duodenal fluid and M17 broth depended significantly on the strain (p-value = 0.0001) and the environment (p-value = 0.0001). The initial number of bacteria ranged from 6.7 log CFU/mL to 7.2 log CFU/mL (Table 4). After a 6 h incubation, the population of *S. thermophilus* was reduced to 2.6-3.8 log CFU/mL, and the viability was 43.8-

Table 4. The viability of *Streptococcus thermophilus* isolates in artificial duodenal fluid and M17 broth

| Isolate number | Initial number of bacteria log CFU/mL | Artificial duodenal fluid | | | M17 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 ±0.2 | 3.0 ±0.2 | 3.8 ±0.2 a | 43.8 ±2.4 a | 6.9 ±0.1 | –0.1 ±0.2 a | 101.5 ±2.8 a |
| 2 | 6.9 ±0.1 | 3.1 ±0.2 | 3.8 ±0.2 a | 45.5 ±2.6 a | 6.8 ±0.2 | 0.1 ±0.1 ab | 99.1 ±1.9 ab |
| 3 | 6.8 ±0.2 | 3.2 ±0.2 | 3.7 ±0.4 a | 46.6 ±4.3 ab | 6.9 ±0.1 | 0.0 ±0.1 ab | 100.3 ±1.9 ab |
| 4 | 7.1 ±0.3 | 3.3 ±0.3 | 3.8 ±0.6 a | 46.4 ±6.0 ab | 6.9 ±0.2 | 0.1 ±0.2 ab | 98.1 ±2.3 ab |
| 5 | 6.7 ±0.2 | 4.1 ±0.1 | 2.7 ±0.2 b | 60.4 ±1.6 de | 6.8 ±0.1 | –0.1 ±0.1 a | 101.2 ±2.0 a |
| 6 | 6.8 ±0.2 | 3.9 ±0.1 | 2.9 ±0.2 b | 57.7 ±1.3 de | 6.9 ±0.1 | –0.1 ±0.2 ab | 101.0 ±2.8 ab |
| 7 | 6.9 ±0.4 | 3.8 ±0.2 | 3.1 ±0.6 bc | 55.8 ±6.0 cd | 6.7 ±0.3 | 0.2 ±0.4 b | 97.6 ±5.3 b |
| 8 | 7.2 ±0.3 | 3.6 ±0.3 | 3.5 ±0.2 ac | 50.8 ±2.0 bc | 7.1 ±0.2 | 0.1 ±0.2 ab | 99.0 ±2.3 ab |
| 9 | 6.9 ±0.4 | 4.1 ±0.1 | 2.9 ±0.4 b | 58.9 ±2.8 de | 7.0 ±0.2 | 0.0 ±0.3 ab | 100.5 ±3.8 ab |
| 10 | 7.0 ±0.4 | 4.2 ±0.1 | 2.8 ±0.3 b | 60.6 ±2.8 de | 6.9 ±0.2 | 0.1 ±0.2 ab | 99.0 ±2.5 ab |
| 11 | 7.0 ±0.5 | 4.3 ±0.2 | 2.7 ±0.7 b | 61.4 ±6.8 e | 7.0 ±0.3 | 0.1 ±0.2 ab | 99.0 ±2.5 ab |
| 12 | 6.8 ±0.2 | 4.2 ±0.2 | 2.6 ±0.4 b | 61.8 ±4.5 e | 6.9 ±0.2 | 0.0 ±0.1 ab | 100.6 ±1.9 ab |

Results are expressed as mean ±standard deviation, n = 5.
Different letters in the same column indicate significant differences between means with Tuckey test ($p < 0.05$).

-61.8%. Simultaneously, the final number of bacteria in M17 broth was 6.7-7.1 log CFU/mL, and viability ranged from 97.6% to 101.5%.

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.

## DISCUSSION

Some of the *in vitro* cholesterol uptake results for *S. thermophilus* are 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 LAB and bifidobacteria in MRS broth during an 18 h culture at 37°C. Two cultures of *S. thermophilus* removed 69 and 59 µg/mL of cholesterol. Ziarno et al. [2007] studied the ability of selected thermophilic LAB to assimilate cholesterol during a 24 h culture in MRS broth. Traditional yoghurt starter cultures (consisting of *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*) assimilated less than 27% of the initial cholesterol (700 µg/mL). These studies have demonstrated high values of cholesterol removal by thermophilic LAB, including

*S. thermophilus*. However, while bacteria were incubated for 18 or 24 h in MRS broth, in the present study the incubation period was 5 and 6 h in M17 broth.

There is no scientific information in the literature to indicate that cholesterol is taken up by *S. thermophilus* from artificial GIT fluids. Previously studied *Lactobacillus* cultures were characterized as having a strong ability to uptake cholesterol during growth in MRS broth, artificial gastric fluid, and artificial duodenal fluid [Ziarno et al. 2007, Ziarno 2009]. All *Lactobacillus* cultures removed much more cholesterol from artificial duodenal fluid than from gastric fluid, but much less than from MRS broth. The percentage of cholesterol removed by *Lb. delbrueckii* subsp. *bulgaricus* from artificial gastric fluid was 0.5-5.6% (initially 560 µg/mL). Simultaneously, the amount of cholesterol removed from MRS broth was 4.5-15.4%. Only two isolates removed significantly more cholesterol from MRS broth than from the duodenal fluid. In comparison, the amount of cholesterol removed from artificial duodenal fluid was 20-78 µg/mL [Ziarno 2009]. In the present study, *S. thermophilus* isolates showed the ability to remove cholesterol, and the degree of cholesterol uptake was dependent on the isolate and the incubation environment. Higher cholesterol uptake was observed during incubation in laboratory medium (M17 broth). *S. thermophilus* isolates removed less cholesterol from artificial duodenal fluid than from M17 broth. No reduction of cholesterol level was observed in artificial gastric fluid. This could suggest that the incubation environment with neutral acidity is more beneficial for cholesterol uptake than an acidic environment. These data confirmed the observations made by Lin and Chen [2000] and Ziarno [2009] regarding lactobacilli cultures.

Survival of the GIT is one of the preconditions for LAB to develop any beneficial effects after their consumption. It is necessary that potential probiotic bacteria survive the low pH values of the stomach and tolerate the bile salts in the duodenum. *S. thermophilus* is one of the two most important organisms used in yoghurt production, and it is commonly believed that this bacterium is not bile tolerant and thus is unable to grow or survive in the intestine. However, several authors have evaluated the capability of *S. thermophilus* cells to survive and proliferate in the human intestine and have obtained contradictory results [Tanaka et al. 1999, Brigidi et al. 2003, Campo et al. 2005, Mater et al. 2005, 2006, Elli et al. 2006].

Conway et al. [1987] studied *in vivo* and *in vitro* survival of *S. thermophilus* cultures when exposed to human gastric juice and the effects of an additive, such as milk, on their survival. At pH 3.0 the viable count of bacterial cells decreased to < 3 log CFU/mL after 1.0-1.5 h. The presence of skim milk raised the pH and increased the survival of all strains.

Results obtained by Tanaka et al. [1999] confirmed that some strains of *S. thermophilus* showed bile salt hydrolase (BSH) activity. This could suggest that *S. thermophilus* is able to survive in the intestine. The ability of *Streptococcus thermophilus* administered in yoghurt to survive the passage through the upper gastrointestinal tract was investigated with Gottingen minipigs by Lick et al. [2001]. Living *S. thermophilus* cells were detected in the magnitude of 6-7 log CFU/g of intestinal contents in all animals after 3-6 h post ingestion. They decreased rapidly after 8 h. The study carried out by Elli et al. [2006] included 20 healthy males and females, which ate 125 g of commercial yoghurt twice a day for 1 week. The daily intake of bacteria from yoghurt, containing $2.4 \cdot 10^7$ CFU/g of *Lb. delbrueckii* subsp. *bulgaricus* and $2.0 \cdot 10^8$ CFU/g of *S. thermophilus*, was about $6 \cdot 10^9$ CFU of *Lb. delbrueckii* subsp. *bulgaricus* and $5 \cdot 10^{10}$ CFU of *S. thermophilus*. Yoghurt bacteria were found in the faecal samples, which were

obtained at the beginning of each trial (time zero) and after 2 and 7 days. Mater et al. [2006] used an *in vivo* experiment with gnotoxenic and human-microbiota-associated mouse models. In the gnotoxenic mice, *S. thermophilus* populations increased between 0.5-2.5 h post-inoculation in the small intestine and the caecum-colon. After 2.5 h, the bacterial populations reached a plateau in the caecum-colon (6-7 log CFU/g digestive content). In comparison, the bacterial populations reached undetectable levels after 10 h in the small intestine. This means the *S. thermophilus* survival rate was near 100% in the caecum-colon throughout the experiment and was strongly reduced to less than 0.2% in the small intestine at 10 h. With human-microbiota-associated mice, *S. thermophilus* populations in the small intestine were maximal as early as 0.5 h post-inoculation (7-8 log CFU/g digestive content) and remained at this level for about 1 h before starting to decrease.

Cholesterol uptake and survival during passage through the human GIT are generally considered key features for probiotics to preserve their expected health-promoting effects. The present study confirmed that *S. thermophilus* isolated from yoghurt starter cultures possessed some survival ability in artificial GIT fluids. The previous study regarding the ability of lactic acid bacteria cells to survive in artificial GIT fluids showed differences among the species and genera of LAB [Ziarno 2007, 2009]. These results suggest that *S. thermophilus* isolates survived in duodenal fluid, and could reduce cholesterol levels in the intestinal compartment. Nevertheless, the application of the term "probiotic" to some strains of *S. thermophilus* is still under discussion [Weid et al. 2001, Guarner et al. 2005]. This suggests that the possible effect of living bacteria contained in yoghurt on human organism has not been sufficiently explored.

## CONCLUSIONS

1. The ability of *S. thermophilus* cells to survive in artificial gastric fluid and artificial duodenal fluid varied according to the isolates.
2. Isolates of *S. thermophilus* did not remove statistically significant amounts of cholesterol from artificial gastric fluid.
3. *S. thermophilus* isolates showed the ability to uptake cholesterol from M17 broth and artificial duodenal fluid, and the degree of cholesterol uptake depended on the isolate. All isolates of *S. thermophilus* remove much more cholesterol from M17 broth than from artificial duodenal fluid.
4. All *S. thermophilus* isolates had worse survival in artificial gastric or duodenal fluids than in M17 broth.

## REFERENCES

Agerholm-Larsen L., Bell M.L., Grunwald G.K., Astrup A., 2000. The effect of a probiotic milk product on plasma cholesterol: a meta-analysis of short-term intervention studies. Eur. J. Clin. Nutr. 54, 11, 856-860.

Akalin A.S., Gonc S., Duzel S., 1997. Influence of yogurt and acidophilus yogurt on serum cholesterol levels in mice. J. Dairy Sci. 80, 11, 2721-2725.

Benkerroum N., Oubel H., Mimoun L.B., 2002. Behavior of *Listeria monocytogenes* and *Staphylococcus aureus* in yogurt fermented with a bacteriocin-producing thermophilic starter. J. Food Prot. 65, 5, 799-805.

Bezkorovainy A., 2001. Probiotics: determinants of survival and growth in the gut. Am. J. Clin. Nutr. 73, 2, 399S-405S.

Brigidi P., Swennen E., Vitali B., Rossi M., Matteuzzi D., 2003. PCR detection of *Bifidobacterium* strains and *Streptococcus thermophilus* in feces of human subjects after oral bacteriotherapy and yogurt consumption. Int. J. Food Microbiol. 81, 3, 203-209.

Conway P.L., Gorbach S.L., Goldin B.R., 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J. Dairy Sci. 70, 1-12.

Campo del R., Bravo D., Cantón R., Ruiz-Garbajosa P., García-Albiach R., Montesi-Libois A., Yuste F. J., Abraira V., Baquero F., 2005. Scarce evidence of yogurt lactic acid bacteria in human feces after daily yogurt consumption by healthy volunteers. Appl. Environ. Microbiol. 71, 547-549.

Clavel T., Carlin F., Lairon D., Nguyen-The C., Schmitt P., 2004. Survival of *Bacillus cereus* spores and vegetative cells in acid media simulating human stomach. J. Appl. Microbiol. 97, 214-219.

Dilmi-Bouras A., 2006. Assimilation (*in vitro*) of cholesterol by yogurt bacteria. Ann. Agric. Environ. Med. 13, 1, 49-53.

Elli M., Callegari M.L., Ferrari S., Bessi E., Cattivelli D., Soldi S., Morelli L., Goupil Feuillerat N., Antoine J.M., 2006. Survival of yogurt bacteria in the human gut. Appl. Environ. Microbiol. 72, 5113-5117.

Gagnaire V., Piot M., Camier B., Vissers J.P., Jan G., Léonil J., 2004. Survey of bacterial proteins released in cheese: a proteomic approach. Int. J. Food Microbiol. 94, 2, 185-201.

Guarner F., Perdigon G., Corthier G., Salminen S., Koletzko B., Morelli L., 2005. Should yoghurt cultures be considered probiotic? Br. J. Nutr. 93, 783-786.

Hassan A.N., Ipsen R., Janzen T., Qvist K.B., 2003. Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. J. Dairy Sci. 86, 5, 1632-1638.

Heyman M., Terpend K., Ménard S., 2005. Effects of specific lactic acid bacteria on the intestinal permeability to macromolecules and the inflammatory condition. Acta Paediatr. Suppl. 94, 449, 34-36.

Hosono A., Tono-Oka T., 1995. Binding of cholesterol with lactic acid bacterial cells. Milchwissenschaft 50, 556-560.

Ivanova I., Miteva V., Stefanova T., Pantev A., Budakov I., Danova S., Moncheva P., Nikolova I., Dousset X., Boyaval P., 1998. Characterization of a bacteriocin produced by *Streptococcus thermophilus* 81. Int. J. Food Microbiol. 42, 3, 147-158.

Kiessling G., Schneider J., Jahreis G., 2002. Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. Eur. J. Clin. Nutr. 56, 9, 843-849.

Kimoto H., Ohmono S., Okamoto T., 2002. Cholesterol removal from media by lactococci. J. Dairy Sci. 85, 3182-3188.

Lick S., Drescher K., Heller K.J., 2001. Survival of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* in the terminal ileum of fistulated Göttingen minipigs. Appl. Environ. Microbiol. 67, 9, 4137-4143.

Lim H.J, Kim S.Y., Lee W.K., 2004. Isolation of cholesterol-lowering lactic acid bacteria from human intestine for probiotic use. J. Vet. Sci. 5, 391-395.

Lin M.Y., Chen T.W., 2000. Reduction of cholesterol by *Lactobacillus acidophilus* in culture broth. J. Food Drug Anal. 8, 97-102.

Marteau P., Minekus M., Havenaar R., Huis In't Veld J.H.J., 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine, validation and the effects of bile. J. Dairy Sci. 80, 1031-1037.

Matalon M.E., Sandine W.E., 1986. *Lactobacillus bulgaricus, Streptococcus thermophilus* and yogurt: a review. Cult. Dairy Prod. J. 21, 6-12.

Mater D.D., Drouault-Holowacz S., Oozeer R., Langella P., Anba J., Corthier G., 2006. Beta--galactosidase production by *Streptococcus thermophilus* is higher in the small intestine than in the caecum of human-microbiota-associated mice after lactose supplementation. Br. J. Nutr. 96, 1, 177-181.

Mater D.D.G., Bretigny L., Firmesse O., Flores M.-J., Mogenet A., Bresson J.-L., Corthier G., 2005. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* survive gastrointestinal transit of healthy volunteers consuming yogurt. FEMS Microbiol. Lett. 250, 185-187.

Medici M., Vinderola C.G., Weill R., Perdigón G., 2005. Effect of fermented milk containing probiotic bacteria in the prevention of an enteroinvasive *Escherichia coli* infection in mice. J. Dairy Res. 72, 2, 243-249.

Menard S., Laharie D., Asensio C., Vidal-Martinez T., Candalh C., Rullier A., Zerbib F., Megraud F., Matysiak-Budnik T., Heyman M., 2005. *Bifidobacterium breve* and *Streptococcus thermophilus* secretion products enhance T helper 1 immune response and intestinal barrier in mice. Exp. Biol. Med. 230, 10, 749-756.

Michel V., Martley F.G., 2001. *Streptococcus thermophilus* in cheddar cheese – production and fate of galactose. J. Dairy Res. 68, 2, 317-325.

Pereira D.I.A., Gibson G.R., 2002. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. Appl. Environ. Microbiol. 68, 4689-4693.

Rasic J.L., Vujicic I.F, Skringer M., Vulic M., 1992. Assimilation of cholesterol by some cultures of lactic acid bacteria and bifidobacteria. Biotechnol. Lett. 14, 39-44.

Shene C., Canquil N., Bravo S., Rubilar M., 2008. Production of the exopolysaccharides by *Streptococcus thermophilus*: effect of growth conditions on fermentation kinetics and intrinsic viscosity. Int. J. Food Microbiol. 124, 3, 279-284.

Tanaka H., Doesburg K., Iwasaki T., Mierau I., 1999. Screening of lactic acid bacteria for bile salt hydrolase activity. J. Dairy Sci. 82, 12, 2530-2535.

Thibault H., Aubert-Jacquin C., Goulet O., 2004. Effects of long-term consumption of a fermented infant formula (with *Bifidobacterium breve* c50 and *Streptococcus thermophilus* 065) on acute diarrhea in healthy infants. J. Pediatr. Gastroenterol. Nutr. 39, 2, 147-152.

Weid Von der T., Donnet-Hughes A., Blum S., Schiffrin E.J., Neeser J.R., Pfeifer A., 2001. Scientific thoroughness of human studies showing immunestimulating properties of yogurt. Am. J. Clin. Nutr. 73, 133-134.

Ziarno M., 2007. Survival of lactic acid bacteria in simulated duodenal fluid depending on the cholesterol presence. Pol. J. Food Nutr. Sci. 57, 4C, 625-631.

Ziarno M., 2009. *In vitro* cholesterol uptake by *Lactobacillus delbrueckii* subsp. *bulgaricus* isolates. Acta Sci. Pol., Technol. Aliment. 8, 2, 21-32.

Ziarno M., Sekul E., Lafraya Aguado A., 2007. Cholesterol assimilation by commercial yoghurt starter cultures. Acta Sci. Pol., Technol. Aliment. 6, 1, 83-94.

Zourari A., Accolas J.P., Desmazeaud M.J., 1992. Metabolism and biochemical characteristics of yogurt bacteria: a review. Lait 72, 1-34.

## PRZEŻYWALNOŚĆ I USUWANIE CHOLESTEROLU PRZEZ KULTURY *STREPTOCOCCUS THERMOPHILUS* W WARUNKACH MODELOWYCH SOKÓW TRAWIENNYCH

**Wstęp.** Bakterie z gatunku *Streptococcus thermophilus* są stosowane tradycyjnie wraz z *Lb. delbrueckii* subsp. *bulgaricus* jako kultura starterowa w produkcji jogurtów. Niektórzy badacze wskazują, że *S. thermophilus* mogą przynosić dodatkowe korzyści zdrowotne, na przykład obniżać poziom cholesterolu. Celem badań była ocena usuwania choleste-

rolu i przeżywalności izolatów *S. thermophilus* w warunkach *in vitro* w modelowych środowiskach symulujących układ pokarmowy.
**Materiał i metody.** Dwanaście izolatów *S. thermophilus* przetrzymywano w modelowym soku żołądkowym (z dodatkiem pepsyny), w modelowym soku trzustkowym (z dodatkiem enzymów trzustkowych) i w bulionie M17 zawierających cholesterol w początkowym stężeniu 600 µg/ml, oraz w bulionie M17 niezawierającym dodatku cholesterolu. Bezpośrednio po dodaniu inoculum bakteryjnego i po zakończeniu doświadczeń mierzono stężenie cholesterolu w podłożu hodowlanym oraz liczbę komórek bakterii.
**Wyniki.** *S. thermophilus* nie usuwały istotnych ilości cholesterolu z modelowego soku żołądkowego. Wykazały jednak zdolność do usuwania cholesterolu z bulionu M17 i modelowego soku trzustkowego, a ilość usuniętego cholesterolu zależała od izolatu. Wszystkie izolaty *S. thermophilus* usuwały więcej cholesterolu z bulionu M17 niż z modelowego soku trzustkowego. Wszystkie izolaty *S. thermophilus* gorzej przeżywały w modelowym soku żołądkowym lub modelowym soku trzustkowym niż w bulionie M17.
**Wnioski.** Zdolność komórek *S. thermophilus* do przeżywania w modelowym soku żołądkowym i modelowym soku trzustkowym zależała od izolatu.

**Słowa kluczowe:** cholesterol, *Streptococcus thermophilus*, usuwanie cholesterolu, modelowy sok żołądkowy, modelowy sok trzustkowy

*Accepted for print – Zaakceptowano do druku: 6.10.2009*

*For citation – Do cytowania: Ziarno M., 2010. Viability and cholesterol uptake by Streptococcus thermophilus cultures in artificial GIT fluids. Acta Sci. Pol., Technol. Aliment. 9(1), 83-94.*