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CHOLESTEROL ASSIMILATION BY COMMERCIAL YOGHURT STARTER CULTURES

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Abstract. The ability to *in vitro* cholesterol level reduction in laboratory media has been shown for numerous strains of lactic acid bacteria, but not for all strains of lactic bacteria used in the dairy industry. The aim of this work was the determination of the ability of selected thermophilic lactic acid bacteria to cholesterol assimilation during 24 h culture in MRS broth. Commercial starter cultures showed various ability to cholesterol assimilation from laboratory medium. In case of starter cultures used for production of traditional yoghurt, consisting of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, the quantity of assimilated cholesterol did not exceed 27% of its initial contents (0.7 g in 1 dm^3). Starter cultures used for bioyoghurt production, containing also probiotic strains (came from *Lactobacillus acidophilus* species or *Bifidobacterium* genus) assimilated from almost 18% to over 38% of cholesterol. For one monoculture of *Lb. acidophilus*, cholesterol assimilation ability of 49-55% was observed, despite that the number of bacterial cells in this culture was not different from number of bacteria in other cultures.

Key words: cholesterol, dairy starter cultures, assimilation, lactic acid bacteria

INTRODUCTION

The ability to *in vitro* cholesterol level reduction in model culture media has been shown for numerous strains of lactic acid bacteria, such as *Lb. acidophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. casei*, *Lb. gasseri*, *Lb. amylovorus* [Gilliland et al. 1985, Rasic et al. 1992, Walker and Gilliland 1993, Buck and Gilliland 1994, Tahri et al. 1996, Noh et al. 1997, Brashears et al. 1998, Grill et al. 2000, Lin and Chen 2000, Hosono et al. 2002]. Other species of bacteria in which a similar activity was observed are: *Bifidobacterium* (*B. bifidum*, *B. longum*), *Streptococcus* (*Str. salivarius* subsp. *thermophilus*) and *Enterococcus* (*E. faecium*), as well as *Lactococcus* (*Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *lactis* var. *diacetylactis*), *Leuconostoc mesenteroides* subsp. *mesenteroides* [Rasic et al. 1992, Hosono and Tono-Oka 1995, Tahri et al. 1996, Taranto et al. 1996, Dambekodi and Gilliland 1998, Kimoto et al. 2002]. Adhesion of the

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cholesterol to the cell surface and incorporation of the cholesterol into the cellular membrane are the most frequently suggested mechanisms of lactic bacteria activity on cholesterol level [Hosono and Tono-Oka 1995, Noh et al. 1997, Brashears et al. 1998, Dambekodi and Gilliland 1998]. This raised an assumption that similar assimilation phenomenon may also take place in dairy products fermented by lactic bacteria. This seems to be a proof that both probiotic, and traditional strains of lactic acid bacteria included in starter cultures used for production of fermented dairy products possess ability to cholesterol assimilation. However, not all strains of lactic bacteria used in dairy industry have been studied in this respect.

The aim of this work was the determination of the ability of selected thermophilic lactic acid bacteria to cholesterol assimilation during 24 h culture in MRS broth.

MATERIALS AND METHODS

Starter cultures and culture conditions. Five commercial single species and 13 multiple species lyophilized starter cultures used in dairy industry for production of yoghurts and bio-yoghurts were used in this study. Seven first thermophilic cultures contained *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* (Table 1, symbols A-G). Six next cultures were the mixtures of *Bifidobacterium* and other

Table 1. The results of quantitative analysis of culture of traditional yoghurt starter cultures (mean log10 ±standard deviation)
Tabela 1. Wyniki analizy liczby bakterii w tradycyjnych kulturach jogurtowych (średnia wartość logarytmu i odchylenie standardowe)

Starter culture symbol Symbol kultury startero-wej	Qualitative constitu-tion of starter culture Jakościowy skład kultury starterowej	Log10 of number of thermophilic lactobacilli in 1 cm^3 Logarytm liczby termofilnych pałeczek		Log10 of number of thermophilic streptococci in 1 cm^3 Logarytm liczby termofilnych streptokoków	
		MRS without cholesterol MRS bez cholesterolu	MRS with cholesterol MRS z cholesterolem	M17 without cholesterol M17 bez cholesterolu	M17 with choles-terol M17 z cholesterolem
A	*Str. salivarius* subsp. *thermophilus, Lb. delbrueckii* subsp. *bulgaricus*	8.4 ±0.40 a	8.4 ±0.35 a	8.6 ±0.22 A	8.5 ±0.13 A
B		8.9 ±0.47 a	8.8 ±0.40 a	–	–
C		8.4 ±0.44 a	8.5 ±0.31 a	6.8 ±0.66 A	6.9 ±1.05 A
D		8.5 ±0.36 a	8.5 ±0.16 a	–	–
E		8.2 ±0.29 a	8.4 ±0.35 a	–	–
F		8.4 ±0.32 a	8.3 ±0.50 a	–	–
G		7.7 ±0.31 a	7.7 ±0.22 a	7.5 ±0.54 A	7.5 ±0.32 A

– not measured.
a, b, c, ..., A, B, C, ... – means from the same row, with different superscripts, are significant different at $\alpha = 0.05$.
– nie mierzono.
a, b, c, ..., A, B, C, ... – różne indeksy przy średnich z tego samego wiersza oznaczają statystycznie istotna różnicę między tymi średnimi dla $\alpha = 0,05$.

thermophilic lactic acid bacteria (Table 2 and 3, symbols H-M). Last five cultures were the following monocultures: *Bifidobacterium bifidum* (culture N in Table 3), *Bifidobacterium species* (culture O in Table 3), *Lb. acidophilus* (cultures P and Q in Table 3), *Str. salivarius* subsp. *thermophilus* (culture R in Table 3).

Table 2. The results of quantitative analysis of culture of yoghurt starter cultures contained probiotic strains (mean log10 ±standard deviation)
Tabela 2. Wyniki analizy liczby bakterii w kulturach jogurtowych zawierających szczepy probiotyczne (średnia wartość logarytmu i odchylenie standardowe)

Starter culture symbol Symbol kultury startero-wej	Qualitative constitution of starter culture Jakościowy skład kultury starterowej	Log10 of number of thermophilic lactobacilli in 1 cm^3 Logarytm liczby termofilnych pałeczek		Log10 of number of thermophilic streptococci in 1 cm^3 Logarytm liczby termofilnych streptokoków	
		MRS without cholesterol MRS bez cholesterolu	MRS with cholesterol MRS z cholesterolem	MRS without cholesterol MRS bez cholesterolu	MRS with cholesterol MRS z cholesterolem
H	*Str. salivarius* subsp. *thermophilus*, *Lb. acidophilus*, *Bifidobacterium lactis*	8.2 ±0.55 a	8.1 ±0.42 a	8.3 ±0.57 A	8.2 ±0.39 A
I	*Str. salivarius* subsp. *thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Bifidobacterium species*	8.1 ±0.41 a	8.0 ±0.34 a	8.5 ±0.51 A	8.2 ±0.58 A
J		8.0 ±0.50 a	7.9 ±0.47 a	8.3 ±0.61 A	8.0 ±0.44 A
K		8.0 ±0.71 a	8.0 ±0.34 a	–	–
L	*Str. salivarius* subsp. *thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Bifidobacterium bifidum*	8.0 ±0.57 a	7.8 ±0.68 a	8.0 ±0.43 A	7.9 ±0.64 A

– not measured.
a, b, c, ..., A, B, C, ... – means from the same row, with different superscripts, are significant different at $\alpha = 0.05$.
– nie mierzono.
a, b, c, ..., A, B, C, ... – różne indeksy przy średnich z tego samego wiersza oznaczają statystycznie istotna różnicę między tymi średnimi dla $\alpha = 0,05$.

The cultures were grown in MRS broth (Merck) in 37°C for 24 hours, then were transferred into a fresh MRS broth portion and were further grown in 37°C for 24 hours, afterwards, then were used for study.

Cholesterol solution preparation. Cholesterol of chemical purity > 99% (Sigma-Aldrich) was hot dissolved in 99% ethanol and Tween 80, mixed in 3:1 ratio. The obtained solution had a concentration of 3.0 g of cholesterol in 1 dm^3. This solution was used in experiments concerning cholesterol assimilation from MRS broth by LAB. The sterilely measured portion of this solution was added to MRS broth in such a quantity, so that the final concentration of the cholesterol was 0.7 g in 1 dm^3 of the basis.

Table 3. The results of quantitative analysis of culture of single and multiple species starter cultures of probiotic strains (mean log10 ±standard deviation)
Tabela 3. Wyniki analizy liczby bakterii w monokulturach i mieszankach kultur szczepów probiotycznych (średnia wartość logarytmu i odchylenie standardowe)

Starter culture symbol Symbol kultury startero-wej	Qualitative constitution of starter culture Jakościowy skład kultury starterowej	Log10 of number of thermophilic lactobacilli in 1 cm^3 Logarytm liczby termofilnych pałeczek		Log10 of number of thermophilic streptococci in 1 cm^3 Logarytm liczby termofilnych streptokoków	
		MRS without cholesterol MRS bez cholesterolu	MRS with cholesterol MRS z cholesterolem	MRS without cholesterol MRS bez cholesterolu	MRS with cholesterol MRS z cholesterolem
M	*Lb. rhamnosus*, *Bifidobacterium species*	8.2 ±0.72 a	7.8 ±0.64 a	–	–
N	*Bifidobacterium bifidum*	8.1 ±0.52 a	7.9 ±0.58 a	–	–
O	*Bifidobacterium species*	7.6 ±0.54 a	7.6 ±0.29 a	–	–
P	*Lb. acidophilus*	7.9 ±0.86 a	7.8 ±0.55 a	–	–
Q		7.8 ±0.49 a	7.8 ±0.27 a	–	–
R	*Str. salivarius* subsp. *thermophilus*	–	–	8.1 ±0.54 A	7.8 ±0.36 A

– not measured.
a, b, c, ..., A, B, C, ... – means from the same row, with different superscripts, are significantly different at $\alpha = 0.05$.
– nie mierzono.
a, b, c, ..., A, B, C, ... – różne indeksy przy średnich z tego samego wiersza oznaczają statystycznie istotna różnicę między tymi średnimi dla $\alpha = 0{,}05$.

Cholesterol assimilation from MRS broth assay. The revived starter cultures' ability for cholesterol assimilation was calculated as loss of its concentration in MRS broth supernatant after the end of culture in 37°C for 24 hours. Cholesterol concentration was assayed with the enzymatic diagnostic test Cholésterol RTU® (BioMérieux). Absorbance was measured with a spectrophotometer SmartSpecTM 3000 (Bio-Rad). Before cholesterol concentration in MRS broth was assayed the content of tubes was centrifuged in ultra-speed centrifuge (12.000 g, 10 min, 4°C) in order to separate bacterial cells biomass and obtain clear MRS broth supernatant. Percentage value of cholesterol assimilation was calculated using the following formula

$$D = 100 - \frac{A - B}{C} \cdot 100, \%$$

where: A – cholesterol concentration in MRS broth containing suspension of bacterial cells and addition of cholesterol solution,
B – cholesterol concentration in MRS broth containing bacterial cells suspension only,
C – cholesterol concentration in MRS broth containing cholesterol solution addition only,
D – cholesterol assimilation, %.

The assay was performed in five-fold repetition.

Number of lactic acid bacteria cells assay. The number of lactobacilli, bifidobacteria and streptococci was assayed with plate method in parallel cultures in MRS broth containing or not containing an addition of cholesterol solution. Inoculated plates were incubated in conditions optimal for a given species: for lactobacilli and bifidobacteria – MRS Agar (Merck), 37°C for 72 hours, anaerobic conditions; for streptococci – M17 Agar (Merck), 37°C for 48 hours, aerobic conditions.

Statistical analysis. Results of assay of lactobacilli and streptococci numbers in MRS broth containing and not containing the cholesterol solution were analysed using Statgraphics 4.1 Plus software.

RESULTS

The studied commercial starter cultures showed various ability to assimilate cholesterol from culture medium. The results of these studies are presented in Figures 1-3. In the case of starter cultures used for production of traditional yoghurt, the quantity of assimilated cholesterol did not exceed 27% of its initial contents. It is worth reminding that such starter cultures contain only two species of thermophilic lactic acid bacteria: *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. For five such starter cultures average cholesterol assimilation value was from almost 12% to over 21% (Fig. 1).

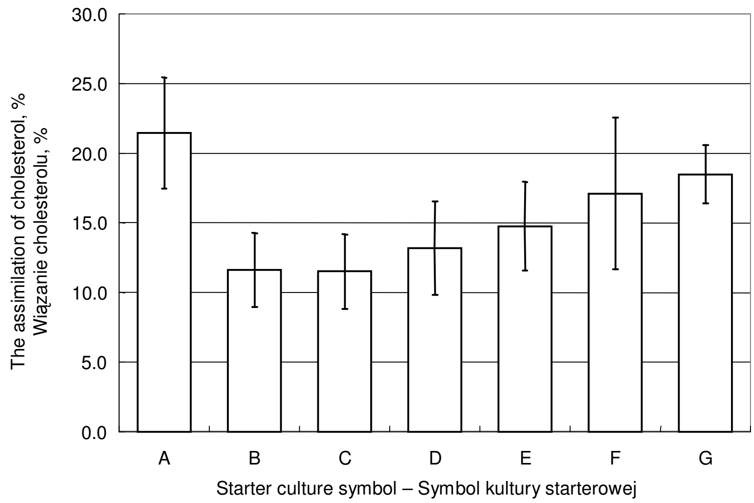

Fig. 1. The assimilation of cholesterol by traditional yoghurt starter cultures (means and standard deviations): A-G – symbols of cultures containing *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*
Rys. 1. Wiązanie cholesterolu przez tradycyjne jogurtowe kultury starterowe (wartości średnie i odchylenia standardowe): A-G – symbole kultur zawierających *Str. salivarius* subsp. *thermophilus* i *Lb. delbrueckii* subsp. *bulgaricus*

This was confirmed also by results of cholesterol assimilation by starter cultures used for bioyoghurt production. Besides two basic yoghurt bacteria species those cultures contain also an additional microflora, for example *Lactobacillus acidophilus* species or *Bifidobacterium* genus. Those starter cultures could assimilate cholesterol at the level of almost 18% to over 38% (Fig. 2), which was significantly higher than for traditional yoghurt cultures.

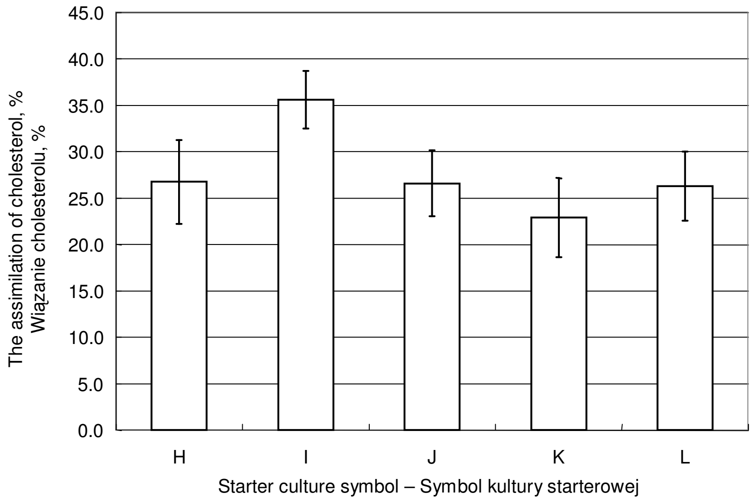

Fig. 2. The assimilation of cholesterol by yoghurt starter cultures containing probiotic strains (means and standard deviations): H – symbol of culture containing *Str. salivarius* subsp. *thermophilus*, *Lb. acidophilus* and *Bifidobacterium lactis*, I-K – symbols of cultures containing *Str. salivarius* subsp. *thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* and *Bifidobacterium species*, L – symbol of culture containing *Str. salivarius* subsp. *thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* and *Bifidobacterium bifidum*

Rys. 2. Wiązanie cholesterolu przez jogurtowe kultury starterowe zawierające szczepy probiotyczne (wartości średnie i odchylenia standardowe): H – symbol kultury zawierającej *Str. salivarius* subsp. *thermophilus*, *Lb. acidophilus* i *Bifidobacterium lactis*, I-K – symbole kultur zawierających *Str. salivarius* subsp. *thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* i *Bifidobacterium species*, L – symbol kultury zawierającej *Str. salivarius* subsp. *thermophilus*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* i *Bifidobacterium bifidum*

Analysis of monocultures or cultures composed of additional microflora only confirmed an assumption that those bacteria possess higher ability of cholesterol assimilation comparing to traditional yoghurt cultures (Fig. 3). One monoculture of *Lb. acidophilus* deserves a special attention. For this culture cholesterol assimilation ability of 49.2-55.4% was observed, despite the number of bacterial cells in this culture was not different from number of bacteria in other cultures (Table 3). Comparably, the monoculture of *Str. salivarius* subsp. *thermophilus* assimilated cholesterol at the level of 23.9% ±6.81% (Fig. 3).

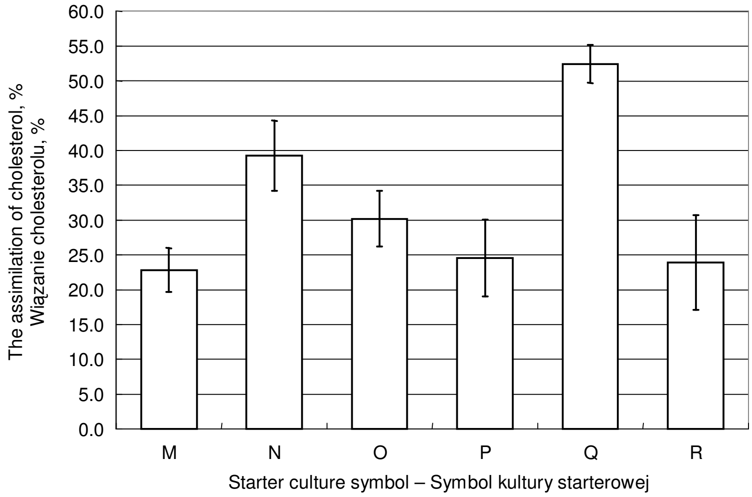

Fig. 3. The assimilation of cholesterol by single and multiple species starter cultures of probiotic strains (means and standard deviations): M – symbol of culture containing *Lb. rhamnosus* and *Bifidobacterium species*, N – symbol of monoculture of *Bifidobacterium bifidum*, O – symbol of monoculture of *Bifidobacterium species*, P-Q – symbols of monocultures of *Lb. acidophilus*, R – symbol of monoculture of *Str. salivarius* subsp. *thermophilus*

Rys. 3. Wiązanie cholesterolu przez monokultury i mieszanki kultur szczepów probiotycznych (wartości średnie i odchylenia standardowe): M – symbol kultury zawierającej *Lb. rhamnosus* i *Bifidobacterium species*, N – symbol monokultury *Bifidobacterium bifidum*, O – symbol monokultury *Bifidobacterium species*, P-Q – symbol monokultury *Lb. acidophilus*, R – symbol monokultury *Str. salivarius* subsp. *thermophilus*

At the same time it was checked if cholesterol solution addition inhibits or stimulates growth of studied cultures. Logarithm of lactobacilli number in cultures was between 6.7 and 9.3, and of streptococci from 5.8 to 9.1. No statistically significant differences were observed both for number of streptococci and number of lactobacilli, between cultures carried in MRS broth with addition of cholesterol solution and without this addition (Table 1-3). Found differences regarded the number of bacteria in cultures of separately studied starter cultures and experiments repetitions.

DISCUSSION

Analysis of culture growth of selected lactic acid bacteria in medium with addition of cholesterol solution and without it, provided in this study, proven lack of significant influence of this addition on the dynamic of those bacteria growth. There is no information in the available literature about any previous attempts to assess this influence. The only exception is the study of Kimoto et al. [2002], who observed that the presence of cholesterol stimulated, to some extent, the growth of cells of *Lc. lactis* subsp. *lactis* biovar *diacetylactis* N7 strain. Kimoto et al. used GM17-THIO broth with 0.2% sodium taurocholate as a medium for bacteria culture and bacterial growth was determined by measuring the optical density of bacterial cells at 620 nm. This method of culture and determination of bacterial growth differed from those used in this work, so it may be a reason for divergent results.

As for the study regarding assessment of lactic acid bacteria ability to assimilate cholesterol there is also no information in literature indicating that commercial starter cultures of lactic acid bacteria have been studied. A large majority of studies referred to in the literature regarded *Lb. acidophilus* strains of intestinal origin (isolated from humans or animals). Monocultures of *Lb. acidophilus* studied in this study, being dairy starter cultures, were characterized by a high ability to assimilate cholesterol during their growth and simultaneous reduction of its concentration in culture medium, comparing to the other studied strains. Value of this assimilation was, however, highly variable depending on repetition.

Results of numerous other studies showed an existence of very significant differences in cholesterol assimilation ability between strains of individual lactic acid bacteria, and frequently even between individual repetitions for the same strain [Gilliland et al. 1985, Gilliland and Walker 1989, Buck and Gilliland 1994, Gopal et al. 1996, Taranto et al. 1996, Pereira and Gibson 2002]. For example, Rasic et al. [1992] observed ability of three *Lb. acidophilus* strains to assimilate cholesterol in MRS broth during 18 h culture in 37°C. The assimilation value for *Lb. acidophilus* strains was from 177 µg to 225 µg in 1 cm^3 of medium. Assimilation values obtained for *Lb. acidophilus* were higher than for other species and strains of lactic acid bacteria studied by those scientists (among others of bifidobacteria, yoghurt cultures of *Str. salivarius* subsp. *thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*). For example, for *Str. salivarius* subsp. *thermophilus* strains the assimilation was only 59 and 69 µg of cholesterol in 1 cm^3 of medium. Walker and Gilliland [1993] found that 11 strains of *Lb. acidophilus* had the ability to assimilate from 0 µmol of cholesterol in 1 cm^3 to 38 µmol cholesterol in 1 cm^3 of MRS THIO medium with an addition of oxgall. Similar observations regarding differences in assimilation between *Lb. acidophilus* strains were made by Gopal et al. [1996]. In their study 6 strains of *Lb. acidophilus* assimilated from 15% to 55% of cholesterol from MRS THIO medium with addition of oxgall.

A complete discussion of the results obtained in this study is hindered by the fact that in the vast majority of published studies, MRS broth medium enriched with bile salts, single (for example sodium taurocholate) or mixture of conjugated or deconjugated bile acid salts (for example oxgall) was used. Also various solutions and concentrations of cholesterol were used, as well as various time of culture. This assumption was confirmed by Lin and Chen [2000], who studied cholesterol level reduction ability of six strains of *Lb. acidophilus* during culture in three different media: one with addition of oxgall (mixture of conjugated and deconjugated bile salts), other with cholic acid (source of deconjugated bile salts), and taurocholic acid (source of conjugated bile salts). The studied strains assimilated from 20% to 57% cholesterol in presence of oxgall, from 43% to 71% cholesterol in presence of cholic acid, and 11-52% cholesterol in presence of taurocholic acid.

Among lactic acid bacteria monocultures studied in this study, high ability of cholesterol assimilation from MRS broth medium during 24 h incubation in 37ºC, was also shown for probiotic monoculture of *Bifidobacterium bifidum* strain (Fig. 3). Active assimilation of cholesterol by cultures of *Bifidobacterium* was also observed by Rasic et al. [1992], who did not find, however, significant differences between the two studied strains of *B. bifidum* (assimilation value of 174 µg of cholesterol in 1 cm^3 and 138 µg of cholesterol in 1 cm^3, respectively). Tahri et al. [1996] observed completely different ability to assimilate cholesterol by two strains of *B. breve.* For *B. breve* ATCC 15700 strain cholesterol assimilation value was 50%, and for *B. breve* ATCC 15698 strain – 9% only. The cited researchers found also that in seven studied strains of bifidobacteria the measured value of assimilation was from 6% (in *B. thermophilum* ATCC 25525 strain) to 50% (in *B. breve* ATCC 15700 strain).

In this study the lowest ability of cholesterol assimilation was observed for bacteria present in typical yoghurt starter cultures being a combination of cultures of *Lb. delbrueckii* subsp. *bulgaricus* and *Str. salivarius* subsp. *thermophilus.* Those results are thus slightly different from those obtained by Rasic et al. [1992]. Lactic acid bacteria from commercial yoghurt culture studied by Rasic et al. bound 111 µg of cholesterol in 1 cm^3 of MRS broth medium during 18 h incubation in 37ºC. One of *Lb. delbrueckii* subsp. *bulgaricus* strain, studied by those researchers showed the highest assimilation ability among all the cultures studied. It was 276 µg of cholesterol in 1 cm^3 of MRS broth. Whereas the studied cultures of *Str. salivarius* subsp. *thermophilus* cultures assimilated less cholesterol comparing to *Lb. delbrueckii* subsp. *bulgaricus* cultures. So, the researchers stated that cultures of thermophilic lactobacilli of *Lb. delbrueckii* subsp. *bulgaricus* species much more actively assimilate cholesterol comparing to cultures of thermophilic streptococci *Str. salivarius* subsp. *thermophilus* species. It must be noted, however, that the ability of cholesterol assimilation may be influenced by numerous factors, such as kind of medium, presence of bile salts, phase of bacterial growth, viability and number of bacterial cells. Hosono and Tono-Oka [1995] and Usman and Hosono [1999] suggest that the differences in the quantity of the assimilated cholesterol are caused by chemical and structural properties of a peptidoglycan present in cellular walls of those bacteria. According to them, this peptidoglycan possessing various amino acid composition in various bacteria, is this component that makes cholesterol attachment (assimilation) to cellular walls possible. However, no studies confirming this hypothesis have been performed yet.

In this study interesting results were obtained in respect to yoghurt starter cultures containing additional microflora *Lb. acidophilus* strains and *Bifidobacterium* species. They confirm a high cholesterol assimilation ability of previously discussed *Lb. acidophilus* strains and *Bifidobacterium* species. Basing on them it is possible to draw a conclusion that those bacteria assimilate cholesterol equally well as monocultures and in presence of other lactic acid bacteria. This is an interesting finding in view of the phenomena of symbiosis or antagonistic growth between bacteria used for yoghurt and bioyoghurt production.

Few literature data suggest ability of some lactic acid bacteria to reduce quantity of cholesterol during production of fermented dairy beverages. This is also confirmed by Polish studies carried out by Juskiewicz and Panfil-Kuncewicz [2003]. The researchers showed that bacteria included in the tested thermophilic starter cultures possess ability to reduce cholesterol contents in milk during its fermentation. Similarly to this study, they observed that cholesterol assimilation level depended on the kind of the used culture and properties of the lactic acid bacteria present in each of those cultures, and also – to a lesser extent – on the fat content in milk. The highest cholesterol assimilation ability was shown by the bacteria present in one classic yoghurt starter culture, for which assimilation value was 22.2% in yoghurt containing 4% fat, and 19.8% on yoghurt containing 8% fat. Another yoghurt starter culture, with similar qualitative composition, showed cholesterol assimilation ability at the level of 11.3% and 15.4%, respectively. In the cited study cholesterol was quite well assimilated also by *Lb. acidophilus* strain (17.5% and 13.6%) and *Bifidobacterium bifidum* strain (12% and 13.5%). Lower cholesterol assimilation activity was shown by *Str. salivarius* subsp. *thermophilus* strain (5.7% and 5.6%). Definitely the weakest cholesterol assimilation was noted by the researchers in bacteria coming from a starter culture containing all the discussed lactic acid bacteria – assimilation value was only 3.1% in yoghurt containing 4% fat and 3.2% in yoghurt containing 8% fat. Thus, a result of the cited study does not overlap the results obtained in this study and concerning the cholesterol assimilation from MRS broth medium. This may be explained by different dynamic of growth of the used lactic acid bacteria in milk and culture medium, and also by other forms of cholesterol present in milk and added to the MRS broth. In milk cholesterol is localized mainly in phospholipids-protein membranes of fat globules, and cholesterol used in experiments of this study was a solution of chemically pure substance.

This additional, potentially health-beneficial effect of thermophilic lactic acid bacteria used in industrial practice constitutes an argument for continuation of this research. It is not known, however, if this cholesterol binding to bacterial cells is stable enough to resist conditions present in human intestinal tract, for example in high acidity, in presence of digestive enzymes of gastric juice or intestine juice. This would explain results of clinical tests showing relationship between consumption of the fermented food products and reduction of cholesterol level in human and animal blood serum.

SUMMARY

1. Commercial yoghurt starter cultures are able to assimilate cholesterol from MRS broth in absence of bile salts. The studied commercial starter cultures showed various abilities to assimilate cholesterol from culture broth.

2. For five typical starter cultures, used for production of traditional yoghurt, the average cholesterol assimilation value was from almost 12% to over 21%. The cholesterol assimilation by starter cultures used for bioyoghurt production was at the level of almost 18% to over 38%.

3. There is a significant difference in cholesterol assimilation ability between strains of individual lactic acid bacteria, and also between individual repetitions for the same strain.

4. The addition of cholesterol solution does not inhibit or stimulate the growth of lactobacilli or streptococci included in commercial yoghurt starter cultures.

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WIĄZANIE CHOLESTEROLU PRZEZ HANDLOWE JOGURTOWE KULTURY STARTEROWE

Streszczenie. U wielu szczepów bakterii fermentacji mlekowej stwierdzono zdolność do wiązania cholesterolu w warunkach *in vitro* w podłożu laboratoryjnym. Celem pracy było zbadanie tej zdolności u handlowych kultur starterowych stosowanych w produkcji jogurtów i biojogurtów. Zdolność do usuwania cholesterolu badano po 24 h hodowli kultur w bulionie MRS w temperaturze optymalnej dla hodowli. Badane kultury starterowe wykazały się różną zdolnością do usuwania cholesterolu z podłoża hodowlanego. W wypadku kultur starterowych stosowanych w produkcji tradycyjnych jogurtów, zawierających

Streptococcus salivarius subsp. *thermophilus* i *Lactobacillus delbrueckii* subsp. *bulgaricus*, ilość usuniętego cholesterolu nie przekraczała 27% początkowej jego zawartości (0,7 g w 1 dm^3). Natomiast u kultur starterowych wykorzystywanych w produkcji biojogurtów, zawierających także szczepy probiotyczne (z gatunków *Lactobacillus acidophilus* lub *Bifidobacterium*), ilość cholesterolu usuniętego wynosiła od 18% do ponad 38%. Dla monokultur *Lb. acidophilus* stopień usunięcia cholesterolu osiągnął 49-55%, pomimo że stwierdzona liczba komórek bakteryjnych w tej kulturze nie różniła się istotnie od ich liczby komórek w innych badanych kulturach.

Słowa kluczowe: cholesterol, mleczarskie kultury starterowe, wiązania cholesterolu, bakterie kwasu mlekowego

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