ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Technol. Aliment. 6(2) 2007, 29-40*

THE INFLUENCE OF CHOLESTEROL AND BIOMASS CONCENTRATION ON THE UPTAKE OF CHOLESTEROL BY *LACTOBACILLUS* FROM MRS BROTH

Małgorzata Ziarno
Warsaw Agricultural University SGGW

Abstract. The aim of this study was the determination of some factors influence (i.e. the vitality of bacteria cells and the cholesterol concentration) on the ability of selected *Lactobacillus* sp. to cholesterol uptake during culture in MRS broth. Three *Lactobacillus* strains (*Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Lb. casei*) isolated from commercial single species lyophilized dairy starter cultures and three *Lactobacillus* strains (*Lb. plantarum*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*) originated from commercial pharmaceuticals were used in this study. The uptake of cholesterol from MRS broth during the growth of *Lactobacillus* sp., expressed as the difference between the final and the initial concentrations of cholesterol, ranged from 0.053 to 0.153 g/dm^3, apart from the initial cholesterol content and the origin of *Lactobacillus* sp. The results confirmed that biomass concentration have a statistically significant effect on uptake of cholesterol. The ten-fold increase of the amount of intact cells biomass caused about 1.5-2-fold increase of the amount of cholesterol removed. The influence of the concentration of biomass of alive cells on the removal of cholesterol was bigger than in case of the heat-sterilized cells.

Key words: cholesterol, dairy starter cultures, cholesterol uptake, *Lactobacillus*, lactic acid bacteria

INTRODUCTION

Several studies on humans have suggested a cholesterol-lowering action of dairy products fermented by lactic acid bacteria. Many *in vitro* reports deal with cholesterol-lowering effects of almost all kinds of lactic acid bacteria. But still, the role of fermented milk products as hypocholesterolemic agents in humans is equivocal, as the clinical studies have given variable data. On the other hand, several *in vitro* studies proposed a number of mechanisms for the cholesterol-lowering action of lactic acid

Corresponding author – Adres do korespondencji: Dr inż. Małgorzata Ziarno, Department of Biotechnology, Microbiology and Food Evaluation of Warsaw Agricultural University SGGW, Nowoursynowska 159 C, 02-776 Warsaw, Poland, e-mail: malgorzata_ziarno@sggw.pl

bacteria. The ability to uptake of cholesterol in laboratory culture media has been shown for numerous *Lactobacillus*, 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]. It has been suggested that sources of variation in hypocholesterolemic effect may be due to the different bacterial strains used in fermentation as well as due to the differences in level of hypocholesterolemic compounds in yoghurt [Jaspers et al. 1984].

Removal of cholesterol by microorganisms present in food could reduce the amount of cholesterol available for human organism. Several studies have indicated that consumption of certain cultured dairy products resulted in reduction of serum cholesterol. This seems to be a proof that probiotic and traditional strains of *Lactobacillus* genera originated from dairy starter cultures or other sources possess ability to cholesterol uptake. However, not all *Lactobacillus* used for human have been studied in this respect. The extension of the range of this study will permit sensitively to qualify, whether and when the process of cholesterol uptake by cells of lactic acid bacteria happens. It will permit also to ascertain, which factors decide about the uptake of cholesterol by bacteria (i.e. pH, temperature, the vitality of bacteria cells, the time of culturing, the secretion of active matters, the cholesterol concentration).

The aim of this study was the determination of some factors influence on the ability of selected *Lactobacillus* sp. to cholesterol uptake during culture in MRS broth.

MATERIALS AND METHODS

Bacterial cultures and culture conditions. Three *Lactobacillus* strains (*Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Lb. casei*) isolated from commercial single species lyophilized dairy starter cultures and three *Lactobacillus* strains (*Lb. plantarum*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*) originated from commercial pharmaceuticals were used in this study. The cultures were grown twice in MRS broth (Merck) in 37°C for 24 h, than were used for study.

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

Influence of the initial concentration of cholesterol on the ability of *Lactobacillus* to uptake of cholesterol. The *Lactobacillus* strains were cultured in MRS broth contained the cholesterol at different initial concentration ranged from 0.083 g/dm^3 to 1.895 g/dm^3. The experiments were performed at 37°C for 24 h. Such culturing conditions made possible the growth of LAB. The experiments have been performed in threefold repetition.

Influence of the concentration of biomass of cells on the ability of *Lactobacillus* to uptake of cholesterol. The *Lactobacillus* strains were cultured in MRS broth contained the cholesterol at known initial concentration. The experiments were performed at 37°C for 24 h. In case of dead biomasses, the cells were heat-sterilized (by autoclaving at 121°C/5 min) and three kind of biomass concentration have been prepared before

the experiments: 10-fold concentrated (10×), normal (1×) and 1-time dissolved (0.1×). The experiments have been performed in three-fold repetition.

Determination of cholesterol content. Cholesterol concentration was assayed with the enzymatic diagnostic test Cholésterol RTU® (BioMérieux). Absorbance was measured with a spectrophotometer Helios Gamma (Thermo Elektron Corporation) at the wavelength 500 nm. Before cholesterol concentration in MRS broth was assayed the content of tubes was centrifuged in ultra-speed centrifuge (6000 r.p.m., 7 min, 4°C) in order to separate bacterial cells biomass and obtain clear MRS broth supernatant. The cultures' ability for cholesterol uptake was calculated as loss of its concentration in MRS broth supernatant after the end of culture. The percent of cholesterol removed from MRS broth by *Lactobacillus* sp. has been expressed as the percent of the initial concentration of cholesterol.

Number of *Lactobacillus* sp. cells. The number of *Lactobacillus* sp. was assayed with plate method. Inoculated plates were incubated in conditions optimal for *Lactobacillus* sp.: MRS Agar (Merck), 37°C for 72 h, anaerobic conditions.

Statistical analysis of results. The statistical analysis of results is accomplished with the help of STATGRAPHICS Plus 5.1 programme. An analysis of variance (one-way or multifactor ANOVA) in order to show the significance of the results has been performed. Since no P-values are less than 0.05, none of the factors have a statistically significant effect on the dependent variable at the 95.0% confidence level.

RESULTS

Cultures of *Lactobacillus* examined in this study showed abilities to remove cholesterol from culture broth which did not depend on the initial concentration of cholesterol (Table 1). This lack of influence of the initial concentration of cholesterol on the uptake of this substance is very interesting. The uptake of cholesterol from MRS broth during the growth of *Lactobacillus* sp., expressed as the difference between the final and the initial concentrations of cholesterol, ranged from 0.053 to 0.153 g/dm^3, apart from the initial cholesterol content and the origin of *Lactobacillus* sp. ($p = 0.2006$). The amount of cholesterol uptake by *Lb. delbrueckii* subsp. *bulgaricus* isolated from dairy starters (0.093 ±0.0214 g/dm^3) did not differ from the amount of cholesterol removed by *Lb. delbrueckii* subsp. *bulgaricus* originated from pharmaceutical (0.089 ±0.0150 g/dm^3). The amount of cholesterol removed by *Lb. acidophilus* isolated from dairy starters (0.091 ±0.0139 g/dm^3) did not differ from the amount of cholesterol uptake by *Lb. acidophilus* originated from pharmaceutical (0.095 ±0.0165 g/dm^3). Only strain of *Lb. casei* removed significantly more cholesterol than *Lb. plantarum* and *Lb. delbrueckii* subsp. *bulgaricus* isolated from pharmaceuticals ($p < 0.05$).

The amount of cholesterol removed from MRS broth, expressed as the percent of the initial concentration of cholesterol, depended on initial cholesterol content ($p < 0.05$) but did not depend on the origin of *Lactobacillus* sp. ($p = 0.2293$). The highest percent of cholesterol uptake has been observed in case of MRS broth containing the lowest initial concentration of cholesterol.

At the same time it was checked if the addition of cholesterol solution inhibits or stimulates growth of *Lactobacillus* sp. (Fig. 1). The initial log of numbers of *Lactobacillus* sp. were 5.20, 6.09, 6.12, 6.33, 6.13 and 6.65 CFU/cm^3 for cultures of *Lb. delbrueckii*

Table 1. The influence of the initial concentration of the cholesterol on the degree of its uptake by chosen *Lactobacillus* (the average and standard deviation from three experiments)
Tabela 1. Wpływ początkowego stężenia cholesterolu na stopień jego związania przez wybrane *Lactobacillus* (średnia i odchylenie standardowe z trzech doświadczeń)

Lactobacillus species Gatunek *Lactobacillus*	Initial concentration of the cholesterol Początkowe stężenie cholesterolu g/dm^3	Amount of removed cholesterol Ilość usuniętego cholesterolu g/dm^3		Percentage of removed cholesterol Procent usuniętego cholesterolu	
		average średnia	SD	average średnia	SD
Lb. bulgaricus (m)	0.120	0.085	0.0150	70.6	12.54
	0.350	0.090	0.0216	25.7	6.18
	0.538	0.085	0.0235	15.9	4.37
	1.020	0.105	0.0338	10.3	3.31
	1.252	0.100	0.0221	8.0	1.77
	1.520	0.093	0.0121	6.1	0.80
Lb. acidophilus (m)	0.090	0.074	0.0072	82.6	8.04
	0.183	0.095	0.0176	51.9	9.61
	0.359	0.079	0.0062	22.0	1.74
	0.751	0.106	0.0192	14.1	2.56
	1.460	0.106	0.0202	7.2	1.38
	1.854	0.085	0.0127	4.6	0.69
Lb. casei (m)	0.118	0.096	0.0071	81.1	6.01
	0.456	0.118	0.0312	25.8	6.84
	0.803	0.098	0.0118	12.2	1.48
	1.201	0.084	0.0079	7.0	0.66
	1.759	0.103	0.0282	5.9	1.60
	1.895	0.110	0.0150	5.8	0.79
Lb. plantarum (f)	0.083	0.068	0.0046	81.9	5.52
	0.330	0.098	0.0090	29.6	2.73
	0.510	0.088	0.0089	17.3	1.74
	0.810	0.086	0.0067	10.7	0.82
	1.143	0.087	0.0300	7.6	2.63
	1.797	0.094	0.0180	5.2	1.00
Lb. bulgaricus (f)	0.125	0.089	0.0075	71.2	6.04
	0.215	0.091	0.0101	42.2	4.68
	0.349	0.078	0.0148	22.4	4.25
	0.742	0.088	0.0078	11.8	1.05
	1.020	0.086	0.0146	8.4	1.44
	1.520	0.100	0.0353	6.6	2.32
Lb. acidophilus (f)	0.154	0.106	0.0154	68.8	10.00
	0.271	0.069	0.0160	25.6	5.91
	0.442	0.103	0.0250	23.2	5.65
	0.649	0.092	0.0104	14.1	1.60
	1.097	0.112	0.0206	10.2	1.88
	1.655	0.086	0.0116	5.2	0.70

Legend: m – cultures originating from commercial dairy starters, f – cultures originating from commercial pharmaceuticals, SD – standard deviation.

Legenda: m – kultury pochodzące z komercyjnych starterów mleczarskich, f – kultury pochodzące z handlowych preparatów farmaceutycznych, SD – odchylenie standardowe.

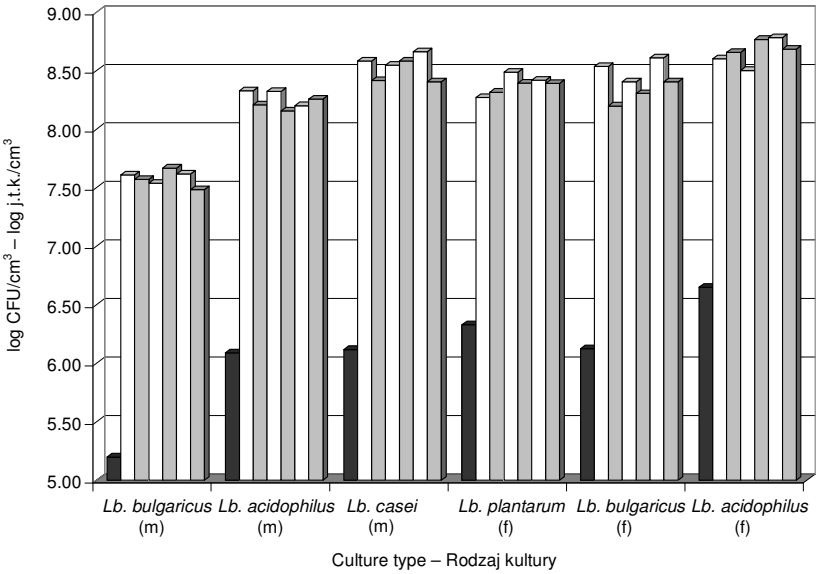

Fig. 1. Growth of *Lactobacillus* strains in MRS broths (the average from three experiments): m – cultures originating from commercial dairy starters, f – cultures originating from commercial pharmaceuticals, darkgrey posts – initial number of lactobacilli, posts grey and white – final number of lactobacilli after incubation at 37°C for 24 h
Rys. 1. Wzrost szczepów *Lactobacillus* w bulionach MRS (średnia z trzech doświadczeń): m – kultury pochodzące z komercyjnych starterów mleczarskich, f – kultury pochodzące z handlowych preparatów farmaceutycznych, słupki ciemnoszare – początkowa liczba pałeczek mlekowych, słupki jasnoszare i białe – końcowa liczba pałeczek po hodowli w temperaturze 37°C przez 24 h

subsp. *bulgaricus*, *Lb. acidophilus* and *Lb. casei* originated from dairy starters, *Lb. plantarum*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* isolated from pharmaceuticals, respectively. No statistically significant differences were observed between the logarithms of *Lactobacillus* sp. number in cultures containing different level of cholesterol solution ($p = 0.9910$, $p = 0.9341$, $p = 0.3786$, $p = 0.9553$, $p = 0.6963$, $p = 0.4853$ for cultures of *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* and *Lb. casei* originated from dairy starters, *Lb. plantarum*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* isolated from pharmaceuticals, respectively).

The influence of the concentration of heat-sterilized biomass on the uptake of cholesterol is presented in the Figure 2. For *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* and *Lb. casei* isolated from starter cultures, the initial cholesterol concentration was 0.350 g/dm^3, 0.810 g/dm^3 and 0.460 g/dm^3 respectively. For *Lb. plantarum*, *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. acidophilus* originated from pharmaceuticals, the initial cholesterol concentration was 0.245 g/dm^3, 0.810 g/dm^3 and 0.460 g/dm^3 respectively. The results confirmed that even cells which were not able to multiply, had ability to remove cholesterol. Statistical analysis confirmed that biomass concentration and type of *Lactobacillus* sp. have a statistically significant effect on uptake of cholesterol at

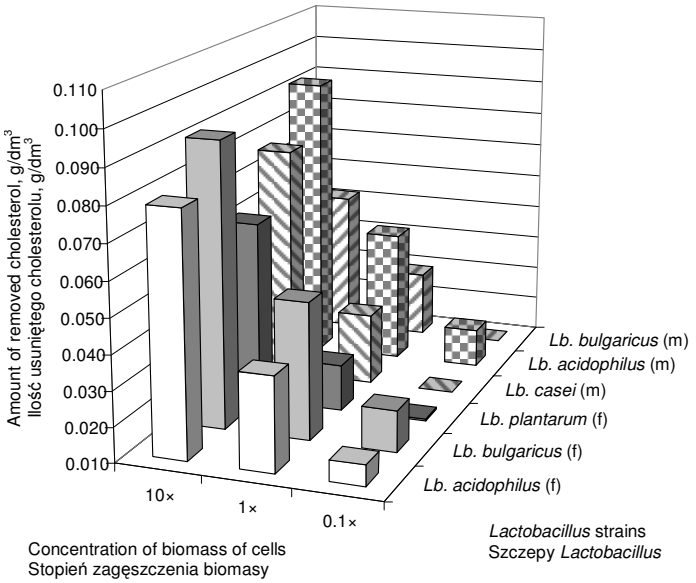

Fig. 2. The influence of the concentration of biomass of dead cells on the uptake of cholesterol by *Lactobacillus* strains (the average from three experiments): m – cultures originating from commercial dairy starters, f – cultures originating from commercial pharmaceuticals
Rys. 2. Wpływ stężenia biomasy martwych komórek na wiązanie cholesterolu przez szczepy *Lactobacillus* (średnia z trzech doświadczeń): m – kultury pochodzące z komercyjnych starterów mleczarskich, f – kultury pochodzące z handlowych preparatów farmaceutycznych

the 95.0% confidence level ($p < 0.05$). The more amount of heat-sterilized biomass the more cholesterol removed. The ten-fold increase of the amount of biomass caused about 1.5-2-fold increase of the amount of cholesterol removed. In case of biomass at normal concentration (1×), the amount of cholesterol removed by heat-sterilized cells ranged from 0.023 ±0.0031 g/dm^3 to 0.049 ±0.0134 g/dm^3 (av. 7.4% ±1.81). But in case of biomass 10×, the amount of cholesterol removed ranged from 0.052 ±0.0055 g/dm^3 to 0.093 ±0.0156 g/dm^3 (av. 16.2% ±2.78). And in case of the lower biomass concentration (0.1×), the amount of cholesterol removed ranged from 0.007 ±0.0055 g/dm^3 to 0.022 ±0.0019 g/dm^3 (av. 5.8% ±1.32). The type of *Lactobacillus* sp. had a statistically significant effect on uptake of cholesterol ($p < 0.05$). *Lb. delbrueckii* subsp. *bulgaricus* originated from dairy starter, *Lb. casei* and *Lb. plantarum* removed statistically less cholesterol than other cultures.

The uptake of cholesterol by alive cells looks totally different. The influence of the concentration of biomass of alive cells on the removal of cholesterol was smaller than in case of the heat-sterilized cells (Fig. 3). For *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* and *Lb. casei* isolated from starter cultures, the initial cholesterol concentration was 0.350 g/dm^3, 0.810 g/dm^3 and 0.460 g/dm^3 respectively. For *Lb. plantarum*, *Lb. delbrueckii* subsp. *bulgaricus* and *Lb. acidophilus* originated from pharmaceuticals, the

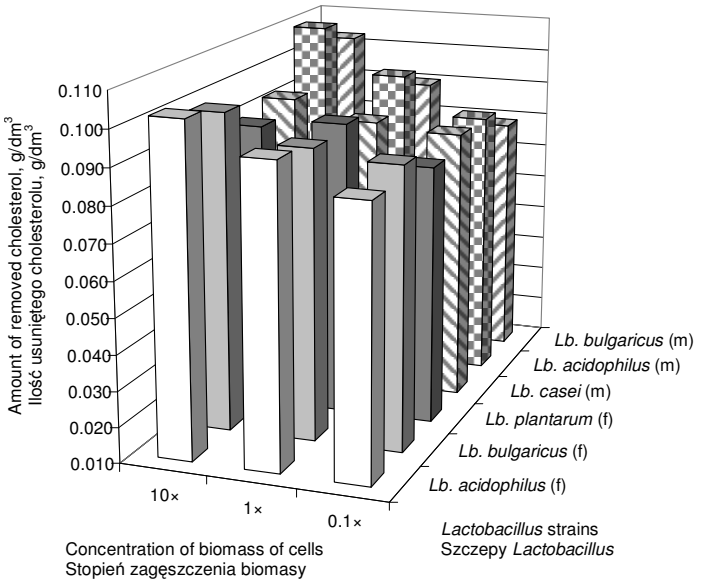

Fig. 3. The influence of the concentration of biomass of alive cells on the uptake of cholesterol by *Lactobacillus* strains (the average from three experiments): m – cultures originating from commercial dairy starters, f – cultures originating from commercial pharmaceuticals
Rys. 3. Wpływ stężenia biomasy żywych komórek na wiązanie cholesterolu przez szczepy *Lactobacillus* (średnia z trzech doświadczeń): m – kultury pochodzące z komercyjnych starterów mleczarskich, f – kultury pochodzące z handlowych preparatów farmaceutycznych

initial cholesterol concentration was 0.245 g/dm^3, 0.810 g/dm^3 and 0.460 g/dm^3 respectively. The amount of cholesterol removed from MRS broth by alive cell, expressed as the difference between the final and the initial concentrations of cholesterol, ranged from 0.079 ±0.0126 g/dm^3 to 0.112 ±0.0007 g/dm^3, apart from the origin of *Lactobacillus* sp. ($p = 0.5936$). The initial biomass concentration had a statistically significant effect on uptake of cholesterol at the 95.0% confidence level ($p = 0.0025$), but this influence was weaker than in case of the heat-sterilized cells. The percent of cholesterol removed from MRS broth, expressed as the percent of the initial concentration of cholesterol, depended on the initial biomass concentration ($p = 0.0298$) and the type of culture ($p = 0.001$). The percent of cholesterol removed by *Lb. delbrueckii* subsp. *bulgaricus* originated from dairy starters (29.4% ±4.31) differed from the percent of cholesterol removed by *Lb. delbrueckii* subsp. *bulgaricus* isolated from pharmaceutical (12.2% ±3.85). The percent of cholesterol removed by *Lb. acidophilus* isolated from dairy starter (13.8% ±0.96) also differed from the percent of cholesterol removed by *Lb. acidophilus* originated from pharmaceutical (22.3% ±1.29). The percent of cholesterol removed by *Lb. casei* and *Lb. plantarum* was 20.3% ±2.25 and 37.9% ±10.41, respectively. The last mentioned value (received for *Lb. plantarum*) gets out of the low the initial content of cholesterol in MRS broth (0.245 g/dm^3) but not of the ability of *Lb. plantarum* used.

DISCUSSION

Monocultures of *Lactobacillus* studied in this study, being isolated from dairy starter cultures and pharmaceuticals, were characterised by high ability to uptake of cholesterol during their growth and simultaneous reduction of its concentration in culture broth.

The value of the uptake of cholesterol represented in the Table 1 confirms that way of the presentation of the results of analyses is very important for appropriate interpretation of the results. It seems that *Lactobacillus* sp. can bind the definite quantity of cholesterol, aside from of this how much it is found in the growth medium.

The results obtained are difficult to discuss due to a lot of study published concerning the uptake of cholesterol by lactic acid bacteria in culture broth in presence of bile salts or bile acids. Many *in vitro* experiments demonstrated that removal of cholesterol from the growth medium results from its precipitation together with deconjugated bile salts and not from its uptake by bacteria [Gilliland et al. 1985, Klaver and Meer 1993, Walker and Gilliland 1993, Brashears et al. 1998, Grill et al. 2000]. Three strains of *Lb. delbrueckii* subsp. *bulgaricus*, studied by Rasic et al. [1992] showed high removal ability: 0.102 ±0.0818 g/dm^3, 0.123 ±0.0507 g/dm^3 and 0.276 ±0.0425 g of cholesterol in 1 dm^3 of MRS broth, respectively. And these values are a little higher that this one received in this study. But Rasic et al. used MRS broth containing 10% PPLO (serum) as a cholesterol source, and bacteria were cultured using 2% inocula for 18 h incubation at 37°C. Cited Authors observed that strains of *Lb. acidophilus* removed significantly more cholesterol at 37°C than strains of *Lb. delbrueckii* subsp. *bulgaricus*, with the exception of one strain of *Lb. delbrueckii* subsp. *bulgaricus*. In comparison, three strains of *Lb. acidophilus* removed 0.177 ±0.0335 g/dm^3, 0.222 ±0.0557 g/dm^3, 0.225 ±0.0453 g of cholesterol in 1 dm^3 of MRS broth.

Also Gilliland and Walker [1989] and Nielsen and Gilliland [1985] reported the different ability to uptake of cholesterol by various strains of *Lb. acidophilus* and *Lb. casei*. This can lead to observed variations in hypocholesterolemic effect of milk products fermented by different bacterial strains.

It should be noticed that the ability to uptake of cholesterol by *Lactobacillus* in MRS broth without bile salts addition is very important from the point of view of food sciences. The obtained results suggested that similar cholesterol removal could take place during food fermentation or cold storage, before that bacteria reach human intestinal tract.

It has been shown that the addition of cholesterol does not inhibit or stimulate growth of *Lactobacillus* sp. (Fig. 1). Previous our study about the growth of selected lactic acid bacteria in media containing the cholesterol solution and without it proven lack of significant influence of this addition to dynamic of those bacteria growth [Ziarno et al. 2006, 2007]. The lack of the influence of the concentrations of cholesterol in MRS broth on the growth of *Lactobacillus* sp. has been shown earlier by us but there is no other information in literature about study like this concerning *Lactobacillus* sp.

Kimoto et al. [2002] observed that presence of cholesterol stimulated to some extent 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.

It must be noted, that ability of cholesterol remove 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. Adhesion of cholesterol to the cell surface and active

incorporation of cholesterol into the cellular membrane are the most frequently suggested mechanisms of lactic bacteria activity on cholesterol level [Hosono and Tonooka 1995, Noh et al. 1997, Brashears et al. 1998]. Hosono and Tonooka [1995] and Usman and Hosono [1999 a] suggest that the differences in quantity of removed 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 compositions in various bacteria is this component that makes cholesterol attachment (assimilation) to cellular walls possible.

The data from many *in vitro* studies show that some strains of *Lactobacillus* are able to take up cholesterol into their cellular membrane [Gilliland et al. 1985, Rasic et al. 1992, Noh et al. 1997, Lin and Chen 2000]. Noh et al. [1997] demonstrated the ability of a strain of *Lb. acidophilus* to incorporate cholesterol from growth broth into cellular membranes during growth. Cholesterol removed by *Lb. acidophilus* ATCC 43121 was not metabolically degraded; most of it was recovered with the cells.

The cell membrane is the primary target for the substances present in the environments of the bacteria. Therefore, its physical properties and fluidity need constant regulation in response to this environment. Taranto et al. [2003] observed that the exposure of *Lb. reuteri* to cholesterol produces changes in the cellular ultra-structure. The cultures growth in presence of cholesterol solution displayed a decrease in glycolipids and a low ratio of saturated:unsaturated fatty acids. The cultures growth in presence of the mixture of cholesterol and bile salts displayed a decrease in phospholipids, glycolipids and also a double ratio of saturated:unsaturated fatty acids. It is possible that cholesterol or bile salts form hydrogen bonds with the hydrogen-accepting groups present in phospholipids and glycolipids. These hydrogen bonds would interfere the binding of cholesterol or bile salts to the lipid fraction, decreasing to a certain extent the effect of cholesterol or bile salts upon the cells. In addition, in case of culture in broth containing cholesterol and bile salts, cholesterol co-precipitated with the deconjugated bile acids but 20% of the initial cholesterol concentration remained tightly bound to the wall/membrane fraction [Taranto et al. 2003]. Therefore it is difficult to compare results of experiences obtained in the presence of bile salts with these effected without this presence.

In present study interesting results were obtained in respect to the concentration and the bioactivity of biomass of cultures of *Lactobacillus*. The results confirmed that even when cell are not able to multiply, the uptake of cholesterol occurs and depends on the amount of bacterial cell biomass. But the degree of the removal of cholesterol is the highest during the growth of bacteria. Aside from of the concentration of the biomass, the alive biomass removed more cholesterol than the biomass of dead cells. In the case of the biomass 10-fold dissolved than 10-fold concentrated greater difference was observed. Comparing the binding of cholesterol by the biomasses of alive cells and the biomasses of the dead cells, the concentration of the alive cells biomass seems to be less important than the concentration of the dead cells biomass.

Usman and Hosono [1999b] showed that the cholesterol-binding ability of two strains of *Lb. gasseri* decreased with increased storage and was related to the number of viable cells. Brashears and Gilliland [1995] suggested that the decrease in viable cells during storage would result in apparent reduced binding of cholesterol.

Comparing Figure 3 and 4 of this study, it can be inferred that the binding of cholesterol by *Lactobacillus* sp. happens with two ways: by adhesion of cholesterol to the surface of dead or alive cells and by incorporation of cholesterol into the cellular mem-

brane of alive cells. In this study the lowest ability of cholesterol uptake was observed for the 10-fold dissolved biomass of the bacteria. The cells from the 10-fold dissolved biomass have a great chance of multiplying as they have less the competition and more of the nutritional components, in proportion to 10-fold concentrated biomass.

The results of this study are compatible with the results obtained by Hosono and Tonooka [1995]. They suspended 10 mg of lyophilized cells of chosen LAB cells in 1 cm^3 of cholesterol-ethanol solution (0.100 mg of cholesterol in 1 ml of 60% ethanol) then the final volume was incubated at 37°C. Their study confirmed that LAB from various fermented milk products was able to remove cholesterol. Binding ability toward cholesterol was observed in all the strains tested even that in these experiments bacteria had not the possibility to multiply. The highest binding ability was observed immediately after incubation was started. It could suggest that binding of cholesterol with intact cells of lactic acid bacteria occurs very quickly and does not need time. Cited Authors showed that intact cells could remove cholesterol at different temperature of incubation (between 10°C and 70°C) and after the autoclaving. The results of heat treatment on the binding activity showed that binding of cholesterol was not largely affected by autoclaving. It is suggested that binding of cholesterol to the bacterial cells may be a physical phenomenon and cell wall related. In described conditions of experiences the binding patterns were not different from those obtained with no heat treated cells. This is conforming to results obtained in this study and concerning the binding abilities of *Lactobacillus* sp. cells which have not the possibility to multiply (were killed or incubated at low temperature).

The observations made in this study are conforming to the results of the experiment carried out by Tabuchi et al. [2004]. They incubated 10 mg of viable cells or heat sterilized cells of *Lactobacillus* GG in one milliliter of cholesterol solution in 60% ethanol (0.100 g/dm^3) at 37°C for 60 min. The ability to decrease in cholesterol *in vitro* was not different between viable *Lb.* GG cells and heat-sterilized *Lb.* GG cells. This observation indicated also that the reaction between *Lb.* GG and cholesterol is due to a direct binding.

The experiments carried out by Tabuchi et al. [2004] suggested that viable cells and heat-sterilized cells might absorb the cholesterol in the fermented dairy products as well as in the small intestine due to their binding action. Cited Authors carried our *in vivo* experiments with the four-week-old male Wistar rats. Rats were fed high-cholesterol diets containing viable *Lb.* GG cells or heat-sterilized *Lb.* GG cells for 14 days. Viable and heat-sterilized *Lb.* GG cells significantly decreased serum total cholesterol and liver cholesterol, more than the rats fed high-cholesterol diet alone. The liver cholesterol was significantly lower in the viable cell group than that in the heat-sterilized cell group. These results indicated that the hypocholesterolemic action of viable *Lb.* GG cells was higher than that of heat-sterilized *Lb.* GG cells.

The results obtained in the present study showed that *Lactobacillus* cells have relatively high cholesterol binding abilities and that the culture conditions are very important for this process. If incorporated into or attached to cells of *Lactobacillus* sp. during growth in the fermented product or in the small intestine, cholesterol is likely to be unavailable for absorption into the human blood. The results of this study concerning the uptake of cholesterol by different *Lactobacillus* sp. cultures may be of interest in selecting desirable strains for the manufacture of dairy starter culture or pharmaceutical. From the present study, it can be concluded that *Lactobacillus* are effective in binding cholesterol *in vitro*. It is also possible that even killed cells of *Lactobacillus* can bind cholesterol in the intestine, when these bacteria and cholesterol are present in diet.

SUMMARY

1. *Lactobacillus* isolates originating from commercial dairy starter cultures and human pharmaceuticals are able to remove cholesterol from MRS broth even in absent of bile salts.

2. Studied *Lactobacillus* isolates showed various abilities to uptake of cholesterol from culture broth, depending on the various factors i.e. biological activity and concentration of bacterial biomass.

3. There is a significant difference in cholesterol uptake ability between dead and alive cells of *Lactobacillus* isolates. The alive cells removed more cholesterol from MRS broth than dead cells did.

REFERENCES

Brashears M.M., Gilliland S.E., 1995. Survival during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* cells as influenced by the growth phase. J. Dairy Sci. 78, 2326--2335.

Brashears M.M., Gilliland S.E., Buck L.M., 1998. Bile salt deconjugation and cholesterol removal from media by *Lactobacillus casei*. J. Dairy Sci. 81, 2103-2110.

Buck L.M., Gilliland S.E., 1994. Comparisons of freshly isolated strains of *Lactobacillus acidophilus* of human intestinal origin for ability to assimilate cholesterol during growth. J. Dairy Sci. 77, 2925-2933.

Gilliland S.E., Nelson C.R., Maxwell C., 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 49, 377-381.

Gilliland S.E., Walker D.K., 1989. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J. Dairy Sci. 73, 905-911.

Grill J.P., Cayuela C., Antoine J.M., Schneider F., 2000. Effects of *Lactobacillus amylovorus* and *Bifidobacterium breve* on cholesterol. Let. Appl. Microbiol. 31, 154-156.

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

Jaspers D.A., Massey L.K., Luedecke L.O., 1984. Effect of consuming yogurts prepared with three culture strains on human serum lipoproteins. J. Food Sci. 49, 1178-1181.

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

Klaver F.A.M., Meer Van Der R., 1993. The assumed assimilation of cholesterol by *Lactobacillus* sp. and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. Appl. Environ. Microbiol. 59, 1120-1124.

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

Nielsen J.W., Gilliland S.E., 1985. Variations in cholesterol assimilation by individual strains of *L. acidophilus* and *L. casei* from human intestines. J. Dairy Sci. 68 (Suppl. 1), 83.

Noh D.O., Kim S.H., Gilliland S.E., 1997. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. J. Dairy Sci. 80, 3107-3113.

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

Tabuchi M., Tamura A., Yamada N., Ishida T., Hosoda M., Hosono A., 2004. Hypocholesterolemic effects of viable and heat-sterilized cells of *Lactobacillus* GG in rats fed a high-cholesterol diet. Milchwissenschaft 59, 249-253.

Tahri K., Grill J.P., Schneider F., 1996. Bifidobacteria strain behaviour toward cholesterol: coprecipitation with bile salts and assimilation. Curr. Microbiol. 33, 187-193.

Taranto M.P., Fernandez Murga M.L., Lorca G., Valdez G.F. de., 2003. Bile salts and cholesterol induce changes in the lipid cell membrane of *Lactobacillus reuteri*. J. Appl. Microbiol. 95, 86-91.

Usman B., Hosono A., 1999 a. Binding of cholesterol the cells and peptidoglycan of *Lactobacillus gasseri*. Milchwissenschaft 54, 495-498.

Usman B., Hosono A., 1999 b. Viability of *Lactobacillus gasseri* and its cholesterol-binding and antimutagenic activities during subsequent refrigerated storage in nonfermented milk. J. Dairy Sci. 82, 2536-2542.

Walker D.R., Gilliland S.E., 1993. Relationship among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. J. Dairy Sci. 76, 956-961.

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

Ziarno M., Sękul E., Makowska M., 2006. Wiązanie cholesterolu przez kultury starterowe mezofilnych paciorkowców mlekowych [The assimilation of cholesterol by starter cultures of mesophilic lactococci]. Biotechnologia 2, 234-246 [in Polish].

WPŁYW STĘŻENIA CHOLESTEROLU I BIOMASY NA USUWANIE CHOLESTEROLU PRZEZ BAKTERIE RODZAJU *LACTOBACILLUS* Z BULIONU MRS

Streszczenie. Celem pracy było określenie wpływu niektórych czynników (początkowego stężenia cholesterolu i żywotności komórek bakteryjnych) na zdolność wybranych pałeczek mlekowych do usuwania cholesterolu w bulionie MRS. W badaniach użyto trzy monokultury pałeczek mlekowych *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Lb. casei* wyizolowane z jednogatunkowych mleczarskich kultur starterowych oraz trzy monokultury pałeczek mlekowych *Lb. plantarum*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* pochodzące z handlowych preparatów farmaceutycznych. Usuwanie cholesterolu bulionu MRS podczas rozwoju pałeczek, wyrażone jako różnica pomiędzy końcowym i początkowym stężeniem cholesterolu w podłożu, wynosiła od 0,053 do 0,153 g/dm^3, niezależnie do początkowego stężenia cholesterolu i pochodzenia monokultur pałeczek. Wyniki potwierdziły, że stężenie biomasy komórkowej statystycznie istotnie wpływa na usunięcie cholesterolu. Dziesięciokrotne zwiększenie ilości biomasy martwych komórek skutkowało około 1,5-2-krotnym wzrostem ilości usuniętego cholesterolu. Wpływ koncentracji biomasy żywych komórek na usuwanie cholesterolu był większy niż w wypadku biomasy komórek zabitych termicznie.

Słowa kluczowe: cholesterol, mleczarskie kultury starterowe, usuwanie cholesterolu, *Lactobacillus*, bakterie fermentacji mlekowej

Accepted for print – Zaakceptowano do druku: 26.03.2007

For citation – Do cytowania: Ziarno M., 2007. The influence of cholesterol and biomass concentration on the uptake of cholesterol by Lactobacillus from MRS broth. Acta Sci. Pol., Technol. Aliment. 6(2), 29-40.