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ASSIMILATION OF CHOLESTEROL BY SOME CULTURES OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA

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SUMMARY

Cultures of Str. thermophilus assimilated less cholesterol than those of Lactobacillus delbrueckii subsp. bulgaricus. A significant difference was found between strains of L. delbrueckii subsp. bulgaricus - LB1 and LB2 and LB3 ($p<0.001$). Bif. bifidum actively assimilated cholesterol, but no significant difference was observed between their two strains ($p>0.05$). Cultures of L. asidophilus assimilated significantly more cholesterol than those of Str. thermophilus and a commercial yoghurt culture.

INTRODUCTION

A cholesterol-lowering effect of fermented milks and their cultures has been the subject of a number of studies. The feeding of a milk formula supplemented with Lactobacillus acidophilus to infants was shown to result in lower levels of serum cholesterol than when the milk without L. acidophilus was fed (Harrison and Peat, 1975). Subsequent reports have shown that rats fed L. acidophilus fermented milk had lower serum cholesterol levels than rats fed plain milk. But, in a second study, there were no significant differences among the groups (Grunewald, 1982 and 1985).

The possible direct action of lactic acid bacteria on cholesterol has been suggested. Various strains of L. acidophilus and L. casei were shown to differ in ability to assimilate cholesterol (Gilliland et al., 1985; Gilliland and Walker, 1990; Nielson and Gilliland, 1985). In studies using pigs on a high cholesterol diet, serum cholesterol was lower when the pigs' diet was supplemented with a strain of L. acidophilus that assimilated cholesterol in vitro, but a strain that did not assimilate cholesterol in a laboratory medium had no effect on serum cholesterol when fed to the pigs.

Reports referring to yoghurt have also indicated variations in hypocholesterolaemic effect. In experiments with rats where the feed included 15% of commercial yoghurt, the levels of total cholesterol, triglycerides and phospholipids were significantly lower in the experimental group than in the control group (Ishida and Kubo, 1985). Similar observations were shown on rabbits fed high-cholesterol feed supplemented with yoghurt or skim milk compared with control-water group (Kiyosawa et al., 1984).

Studies with human subjects receiving 681 g yoghurt daily during three 14-21 day periods have shown an inconsistent effect on serum cholesterol. Three different culture strains were used during studies. It has been suggested that sources of variation in hypocholesterolaemic effect may be due to the different bacterial strains used in fermentation as well as well as due to the differences in level of hypocholesterolaemic compounds in yoghurt (Jaspers et al., 1984).

A few reports deal with cholesterol-lowering effects of other lactic acid bacteria and bifidobacteria. Consumption of milk fermented by Streptococcus thermophilus was shown to result in a significant decrease in serum cholesterol levels of rats compared with the control group (Rao et al., 1981). However, buttermilk added at a rate of 15% of the feed of rats did not affect the serum lipids (Ishida and Fujita, 1987; Ishida and Kub, 1985). The effect of bifidobacteria on cholesterol has been studied with human subjects suffering of elevated serum cholesterol level (300-450 mg%). They received 3 x 2 "Liobif" tablets daily during 6 weeks. Each tablet contained 3 x 10^8 viable cells of Bifidobacterium bifidum. As a results of treatment the levels of serum cholesterol and triglycerides were significantly reduced. In contrast, this treatment had no effect on subjects that had normal cholesterol level (Devečerski et al., 1981). Similar results have been reported in experiments with rats (Homma, 1988).

The aim of this study was to determine variations in ability to assimilate cholesterol in vitro by some cultures of lactic acid bacteria and bifidobacteria.

MATERIALS AND METHODS

Cultures. The following cultures were used in this study: L. delbrueckii subsp. bulgaricus (strains LB1, LB2 and LB3). Str. thermophilus (strains T1 and T2), Bif. bifidum (strains BYU and BPO), L. acidophilus (strains AM, AD and AP), and a commercial yoghurt culture. Bif. bifidum and L. acidophilus were human strains. L. delbrueckii subsp. bulgaricus and Str. thermophilus were obtained from Dairy Research Institute, Belgrade, whereas the strains of Bif. bifidum (BYU and BPO) were obtained from Institute of Virus Research "Torlak", Belgrade.

Strains of L. acidophilus DDS-1, milk-based (AM), DDS-1, vegeterian-based (AD) and adhesion strain (AP) were obtained from Natren, Inc. USA.

The cultures were maintained by routine subculture in sterile skim milk. Each culture was subcultured twice in MRS broth prior to experimental use.

Assimilation of cholesterol. Ability of cultures to assimilate cholesterol was determined using the method described by Gilliland and Walker, 1990, with the exception of MRS broth which was not supplemented with 0.2 % sodium thioglycolate and 0.3 % oxagall. Experimental samples containing 10 % PPLO serum (Difco) as a cholesterol source, were cultured using 2 % inocula for 18 h incubation at 37°C, except Bif. bifidum where 10 % inocula for 20 h incubation were used.

The pH values of samples were determend by using a Metrix glass electrode potentiometer. Analysis for cholesterol was done using the method of Rudel and Morris, 1973. Difference in the quantity of cholesterol in the spent broth samples and in the control uninoculated sample wad taken as quantity of cholesterol assimilated.

Statistical analysis. Analysis of variance was used to determine possible significant variation occuring among means for the different strain cultures of lactic acid bacteria and bifidobacteria.

RESULTS AND DISCUSSION

As shown in Table 1, yoghurt culture strains exihibited variations in assmilation of cholesterol. There was a significant difference between L. delbrueckii subs. bulgaricus, strain LB1 and strains LB2 and LB3 ($p<0.001$). The first culture strain had higher ability to assimilate cholesterol than all the other cultures studied. Generally, no correlation was found between the pH values of cultured MRS broth and choresterol assimilation (Table 2).

Strains of Str. thermophilus assimilated less cholesterol than those of L. delbrueckii subsp. bulgaricus, but the difference between LB2 and T1 and T2 was not significant ($p>0.05$). Commercial yoghurt culture had significantly lower ability of assimilating cholesterol than culture, LB1, but higher ability than Str. thermophilus strains (T1 and T2) and the differences were significant.

Table 1. Assimilation of cholesterol by some cultures of lactic acid bacteria and bifidobacteria during incubation at 37°C[1]

Rank order	Culture[2]	Cholesterol assimilated (µg/ml) Mean[3]	Standard deviation	Rank order[4]										
				1	2	3	4	5	6	7	8	9	10	11
1	LB1	276	42.5	-	NS	2	3	3	3	3	3	3	3	3
2	AP	225	45.3		-	NS	2	3	3	3	3	3	3	3
3	AM	222	55.7			-	NS	NS	2	2	3	2	3	3
4	AD	177	33.6				-	NS	1	2	3	1	3	3
5	BYU	174	45.5					-	NS	1	3	1	3	3
6	BPO	138	47.5						-	NS	NS	NS	2	3
7	LB3	123	50.7							-	NS	NS	1	2
8	Jog.	111	19.9								-	NS	2	3
9	LB2	102	81.8									-	NS	NS
10	T2	69	37.2										-	NS
11	T1	59	36.7											-

[1] MRS broth supplemented with 10 % PPLO serum using 2 % inocula and 18 h incubation, except Bif. bifidum where 10 % inocula and 20h incubation used.

[2] L. delbrueckii subsp. bulgaricus (LB1,LB2,LB3).Str. thermophilus (T1,T2), Bif. bifidum (BYU,BPO), yoghurt culture (Jog.), L. acidophilus (AP, AM, AD).

[3] Each mean is from three trials; and each trial was carried out in triplicate.

[4] NS - not significant difference between means ($p > 0.05$)
1 - significant at $p < 0.05$
2 - significant at $p < 0.01$
3 - significant at $p < 0.001$

Bifidobacteria actively assimilated cholesterol, but no significant difference was found between their strains. However, a significant difference ($p < 0.01$) occurred between strain culture LB1 and bifidobacteria. Strain cultures of L. acidophilus assimilated significantly more cholesterol than those of L. delbrueckii subsp. bulgariacus (LB2 and LB3), with the exception of strain LB1. A significant difference was found between strains LB1 and those of AM and AD, but not between LB1 and AP.

Table 2. Acid production by some cultures of lactic acid bacteria and bifidobacteria in MRS broth supplemented with 10% PPLO serum[1]

Rank order[2]	Culture[3]	pH value
1	BYU	3.70
2	AD	3.80
3	AP	3.85
4	AM	3.85
5	BPO	3.85
6	LB3	4.03
7	LB1	4.06
8	Jog.	4.30
9	LB2	4.35
10	T1	4.40
11	T2	4.45

[1] 2% inocula and 18 h incubation at 37°C, except bifidobacteria where 10% inocula and 20 h incubation at 37°C were used.

[2] According to acid production

[3] L. delbrueckii subsp. bulgaricus (LB1, LB2, LB3), Str. thermophilus (T1, T2), Bif. bifidum (BYU, BPO), yoghurt culture (Jog.), L. acidophilus (AP, AM, AD).

The results of this study showed significant variations in assimilating cholesterol by different strain cultures of yoghurt bacteria and bifidobacteria. Different ability to assimilate cholesterol by various strains of L. adidophilus and L. casei has been earlier reported (Gilliland and Walker, 1990; Nielsen and Gilliland, 1985). Variations in hypocholesterolaemic effect of yoghurt and other fermented milks have been suggested to be at least due to different bacterial strains used in fermentation (Jasper et al., 1984; Rao et al., 1981).

The relatively low ability of Str. thermophilus to assimilate cholesterol found in this study may be due to the rather slow growth of this species in MRS broth. As recognized, Str. thermophilus is a typical milk bacterium and the best medium for its growth is milk (Rao et al., 1981). It is possible that assimilating cholesterol by this organism might be better in vivo.

Our results on the assimilation of cholesterol in vitro by bifidobacteria are in agreement with earlier studies conducted with human subjects, (Devečerski et al., 1981) and with rats, (Homma, 1988), but not with a statement that bifidobacteria may not exhibit hypocholesterolaemic effect (Kim, 1988).

The values for cholesterol assimilation obtained in this study are higher than those reported earlier for L. acidophilus because of different culturing conditions. We used 2 % inocula and 18 h incubation at 37°C, whereas Gilliland and Walker, 1990, used 1 % inocula and 14 or 16 h incubation at 37°C.

The observed variations in assimilating cholesterol by different cultures may be of interest in selecting desirable strains for the manufacture of cultured dairy products.

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