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CHOLESTERAZE ACTIVITY OF NEW LACTO- AND BIFIDOBACTERIA STRAINS *IN VITRO*

Starovoitova S.[1], Kishko K.[1], Lazarenko L.[1], Shynkarenko L.[1], Spivak M.[1], Nikolaychuk M.[2]

Cholesteraze activity of new lacto- and bifidobacteria strains in vitro. — S. Starovoitova[1], K. Kishko[1], L. Lazarenko[1], L. Shynkarenko[1], M. Spivak[1], M. Nikolaychuk[2]. — *Cholesteraze activity of new strains of lacto- and bifidobacteria was studied. It was demonstrated that all strains of lacto- and bifidobacteria in vitro posess different levels of cholesteraze activity. Lactobacillus casei VK-4 IMV B-7280 proved to be the most effective strain. Results of research on cholesteraze activity of different lacto- and bifidobacteria strain compositions showed that the most active combinations are those based on Lactobacillus casei and one of other studied strains of lacto- and bifidobacteria, as well as Bifidobacterium longum and Bifidobacterium bifidum. The studied bacterial strains and also compositions on their basis can be used to create complex probiotics for special purposes.*

Address*: [1] – Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukrain; e-mail: starovoitova_svetlana@svitonline.com ; [2] – Uzhgorod National University, str. A. Voloshina, 32, m. Uzhgorod, Ukraine, 88000.*

Introduction

The results of numerous researches proved that lacto- and bifidobacteria are able to reduce cholesterol level in vivo and in vitro, demonstrating hypocholesterolemiac activity [1–13]. Presumably bacteria are able to directly assimilate cholesterol, bind it by their cellular walls, decompose bilious acids with bacterial enzymes and exert physiological influence on the final components of fatty acids fermentation with short lateral chains (mainly propionate). It should be noted that until now experimental conformation of hypocholesterolemiac activity was achieved only through the above mentioned 3 properties. However, so far no officially renowned medication, based on lacto- and/or bifidobacteria, with sufficient hypocholesterolemiac activity for correction of the increased cholesterol level is known. Development of such a probiotic is a relevant challenge for modern immunobiotechnology.

Research purpose is to establish cholesteraze activity of new lacto- and bifidobacteria strains, and corresponding properties of their compositions by the study of their influence on the level of cholesterol with the purpose of creating on their basis new probiotics with properties designated specifically for chosen purposes.

Materials and methods

The strains of lacto- and bifidobacteria, independently abstracted from the associative culture of the fermented biological material, were a research object: *Bifidobacterium bifidum* VK-1, *Bifidobacterium longum* VK-2, *Lactobacillus acidophilus* VK-3 IMV B-7279, *Lactobacillus casei* VK-4 IMV B-7280, *Lactobacillus bulgaricus* VK-5 IMV B-7281.

To determine bacterial cholesteraze activity *in vitro*, 24-hour cultures of lacto- and bifidobacteria were used, inoculated in MRS broth supplemented with sodium thioglycollate (Sigma), Oxgall (Difco Laboratories) and freshly (ex-tempore, newly) prepared cholesterol (chemical cleanness > 99 %, Sigma-Aldrich, USA) [15]. Bacterial influence on maintenance of cholesterol concentration in MRS broth was determined according to Rudel (1973) after 18 and 24 hours of cultivation.

Results and their discussion

Previously it was shown by us that genus *Lactobacillus* can utilize cholesterol as the unique source of carbon in nourishing broth [18, 19]. But unfortunately the data was only of qualitative character about ability of lactic acid bacteria to reduce the level of cholesterol *in vitro*.

In this study the quantitative indexes of *Lactobacillus* and *Bifidobacterium* cholesteraze activity were determined. Results of new strains are presented in fig. 1 and 2. It was demonstrated that all studied strains of lacto- and bifidobacteria managed to decrease the level of cholesterol in MRS broth both after 18- and 24-hour cultivation.

In case of 18-hour cultivation maximal cholesteraze activity was shown by *Lactobacillus casei* VK-4 IMV B-7280 – 34.40 ± 0.28 %. For strains *Lactobacillus acidophilus* VK-3 IMV B-7279 and *Lactobacillus bulgaricus* VK-5 IMV B-7281 there were approximately identical percents of absorption of cholesterol from the environment of cultivation – 24.73 ± 1.22 and 22.05 ± 0.98 %, accordingly. For the strains of genus *Bifidobacterium* value of cholesteraze activity were following: *Bifidobacterium longum* VK-2 – 17.78 ± 1.21 %; *Bifidobacterium bifidum* VK-1 – 5.38 ± 0.22 %.

24-hour cultivation led to even more significant reduction of cholesterol concentration in the MRS broth (fig. 2). Maximal cholesteraze activity was dem-

onstrated by the strain *Lactobacillus casei* VK-4 IMV B-7280 – 62.37 ± 1.68 % decrease in cholesterol level. The same parameter in case of *Lactobacillus bulgaricus* VK-5 IMV B-7281 and *Lactobacillus acidophilus* VK-3 IMV B-7279 decreased on 38.71 ± 1.21 and 27.96 ± 1.10 % respectively. Amount of eaten up (bound and degraded) cholesterol in the MRS broth by *Bifidobacterium longum* VK-2 and *Bifidobacterium bifidum* VK-1 after 24 hours was 22.46 ± 0.80 and 7.64 ± 0.32 %.

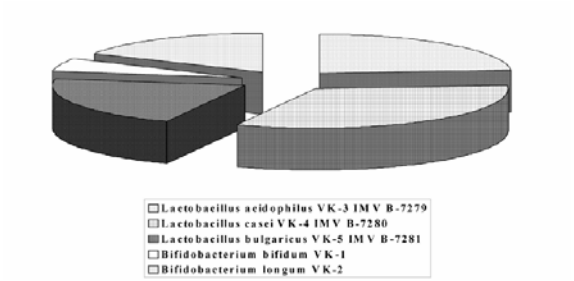

Fig.1. Decreased cholesterol level in MRS broth caused by lacto- and bifidobacteria after 18 hours of cultivation ($P < 0.05$)

Thus, among studied strains the ability to bind cholesterol is not unique, although each strain did have a specific level of cholesteraze activity.

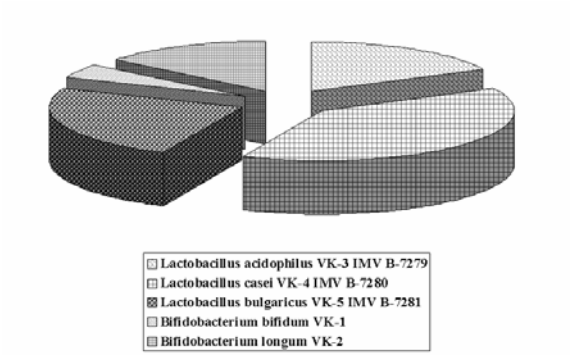

Fig. 2. Reduction of cholesterol in MRS broth under the action of lacto- and bifidobacteria after 24-hour cultivation ($P < 0.05$)

It should be noted that cholesteraze activity of lactic acid bacteria was higher then that of bifidobacteria. The ability of studied strains to reduce cholesterol level in MRS broth decreases in the following sequence: *Lactobacillus casei* VK-4 IMV B-7280 > *Lactobacillus bulgaricus* VK-5 IMV B-7281 > *Lactobacillus acidophilus* VK-3 IMV B-7279 > *Bifidobacterium longum* VK-2 > *Bifidobacterium bifidum* VK-1.

Table 1. Cholesteraze activity of lacto- and bifidobacteria compositions *in vitro*

Composition	Ratio of the strains	Time of cultivation, h.c	Reduction in cholesterol concentration of the MRS broth, %
Lactobacillus casei VK-4 IMB B-7280:*Lactobacillus bulgaricus* VK-5 IMB B-7281:*Lactobacillus acidophilus* VK-3 IMB B-7279: *Bifidobacterium longum* VK-2: *Bifidobacterium bifidum* VK-1	1:1:1:1:1	18	28.17±0.01*
		24	38.03 ± 0.02*
Lactobacillus casei VK-4 IMB B-7280: *Lactobacillus bulgaricus* VK-5 IMB B-7281	1:1	18	33.80 ± 0.03
		24	73.24 ± 0.37
Bifidobacterium longum VK-2: *Bifidobacterium bifidum* VK-1	1:1	18	54.23 ± 0.28*
		24	68.87 ± 0.36*
Lactobacillus casei VK-4 IMB B-7280: *Bifidobacterium bifidum* VK-1	1:1	18	49.30 ± 0.21*
		24	52.82 ± 0.23*
Lactobacillus bulgaricus VK-5 IMB B-7281: *Bifidobacterium longum* VK-2	1:1	18	14.79 ± 0.21*
		24	23.15 ± 0.03*
Lactobacillus casei VK-4 IMB B-7280: *Bifidobacterium longum* VK-2	1:1	18	43.94 ± 0.08*
		24	57.75 ± 0.18*
Lactobacillus acidophilus VK-3 IMB B-7279: *Lactobacillus casei* VK-4 IMB B-7280	1:1	18	32.88 ± 0.19*
		24	64.92 ± 0.07*
Lactobacillus acidophilus VK-3 IMB B-7279: *Lactobacillus bulgaricus* VK-5 IMB B-7281	1:1	18	20.15 ± 0.11*
		24	26.78 ± 0.26*
Lactobacillus acidophilus VK-3 IMB B-7279: *Bifidobacterium longum* VK-2	1:1	18	18.92 ± 0.25*
		24	20.11 ± 0.15*
Lactobacillus acidophilus VK-3 IMB B-7279: *Bifidobacterium bifidum* VK-1	1:1	18	12.07 ± 0.08*
		24	16.16 ± 0.22*
Lactobacillus bulgaricus VK-5 IMB B-7281: *Bifidobacterium bifidum* VK-1	1:1	18	0
		24	0

Note: * – $P < 0,05$ in relation to composition of *Lactobacillus casei* VK-4 IMB B-7280: *Lactobacillus bulgaricus* VK-5 IMB B-7281

It was experimentally established by many works [18] that the different strains of lacto- and bifidobacteria are able to increase their beneficial properties if applied in combination with other probiotics. On this basis, determination of cholesteraze activity of different lacto- and bifidobacteria compositions was the next

stage of the experiment. The obtained data are presented in table 1.

From the data of table 1 we can see that among all studied compositions only one – *Lactobacillus bulgaricus* VK-5 IMB B-7281: *Bifidobacterium bifidum* VK-1 did not quite show cholesteraze activity under the condition of this experiment.

Composition consisting of all five studied lacto- and bifidobacteria strains had an average value of cholesteraze activity.

For compositions: *Lactobacillus acidophilus* VK-3 IMV B-7279 and *Lactobacillus bulgaricus* VK-5 IMV B-7281, *Lactobacillus acidophilus* VK-3 SMV B-7279 and *Bifidobacterium longum* VK-2, *Lactobacillus acidophilus* VK-3 IMV B-7279 and *Bifidobacterium bifidum* VK-1, *Lactobacillus bulgaricus* VK-5 IMV B-7281 and *Bifidobacterium longum* VK-2 cholesteraze activity was lower than the level of cholesterol absorption after 18 hours. It's range was: 12,0 - 20,15 %, and after 24 hours- 16.16 – 26.78 %.

Compositions of *Lactobacillus casei* VK-4 IMV B-7280 and *Bifidobacterium bifidum* VK-1, *Lactobacillus casei* VK-4 IMV B-7280 and *Bifidobacterium longum* VK-2, *Lactobacillus casei* VK-4 IMV B-7280: *Lactobacillus bulgaricus* VK-5 IMV B-7281, and also *Lactobacillus acidophilus* VK-3 IMV B-7279 and *Lactobacillus casei* VK-4 IMV B-7280 demonstrated the highest levels of cholesteraze activity. It should be noted that combination of *Lactobacillus acidophilus* VK-3 IMV B-7279: *Lactobacillus casei* VK-4 IMV B-7280 both after 18- and 24-hour cultivation posessed cholesteraze activity at a similar level with that of *Lactobacillus casei* VK-4 IMV B-7280 strain alone. At the same time each strains of lactic acid bacteria, constituting the composition *Lactobacillus casei* VK-4 IMV B-7280 and *Lactobacillus bulgaricus* VK-5 IMV B-7281, showed high cholesteraze activity and at separate cultivation. Joint cultivation of these strains achieved only a 9 % increase in cholesterol-binding effect after 24 hours of cultivations, but after 18 hours values of cholesteraze activity for joint and separate strains had no significant differences.

The substantial increase of cholesteraze activity for composition *Bifidobacterium longum* VK-2 and *Bifidobacterium bifidum* VK-1 was quite unexpected. It is important to mark that for this bacteria composition cholesteraze activity after 18- and 24-hour cultivations exceeded in 2–10 times the corresponding values of separate strains. It can be assumed that the studied strains of bifidobacteria in case of joint cultivation are able to co-stimulate the cholesterol-binding properties of each other *in vitro*, although it is possible that there are several mechanisms of such synergism.

Thus, among all new studied strains of lacto- and bifidobacteria the highest cholesteraze activity *in vitro* was demonstrated by *Lactobacillus casei* VK-4 IMV B-7280. Rather high cholesteraze activity *in vitro* characterized bacteria composition created on the basis of *Lactobacillus casei* VK-4 IMV B-7280 and one of the other studied lacto- or bifidobacteria strains. And only for one combination: *Lactobacillus acidophilus* VK-3 IMV B-7279 and *Lactobacillus casei* VK-4 IMV B-7280 joint cultivation did not led to higher cholesteraze activity, compared to a corresponding parameter of separately cultivated *Lactobacillus casei* VK-4 IMV B-7280. The strains *Bifidobacterium longum* VK-2 and *Bifidobacterium bifidum* VK-1 are individually characterized as low cholesterol-binders *in vitro*. At the same time joint cultivation of these bacteria led to substantial increase of their cholesteraze effectiveness.

The results of the research suggest that for creation of complex probiotic with cholesteraze activity the most perspective compositions of lacto- and bifidobacteria are: *Bifidobacterium longum* VK-2 and *Bifidobacterium bifidum* VK-1, *Lactobacillus casei* VK-4 IMV B-7280 and *Bifidobacterium bifidum* VK-1, *Lactobacillus casei* VK-4 IMV B-7280 and *Bifidobacterium longum* VK-2, *Lactobacillus casei* VK-4 IMV B-7280 and *Lactobacillus bulgaricus* VK-5 IMV B-7281, as well as *Lactobacillus casei* VK-4 IMV B-7280 and *Lactobacillus acidophilus* VK-3 IMV B-7279.

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