PROBIOTIC STRAINS WITH HYPOCHOLESTEROLEMIC ACTIVITY AS POTENTIAL BASIS OF FUNCTIONAL FOOD PRODUCTS FOR PREVENTION AND CONCOMITANT TREATMENT OF PATHOLOGICAL CONDITIONS ASSOCIATED WITH HIGH CHOLESTEROL LEVELS

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Microbiota plays a key role not only in metabolic functions, but also in their optimization and regulation. In particular, many modern studies confirm the involvement of the microbiome in preventing disorders of lipid metabolism (obesity, hyper- and dyslipidemia), etc. Representatives of obligate microflora provide hypolipidemic and hypotensive effects by deconjugating bile acid salts, assimilating and precipitating cholesterol, reducing the activity of tissue angiotensin-converting enzyme.

The participation of the colonic microflora in cholesterol metabolism is very important. Intestinal microorganisms metabolize cholesterol into coprostanol and further to coprostanone. Acetate and propionate, which are formed during the vital activity of most sucrolytic anaerobes, are absorbed into the blood and, reaching the liver, can affect the synthesis of cholesterol. It has been proven that acetate stimulates cholesterol synthesis, and propionate inhibits it.

Several mechanisms of involvement of the microbiome in maintaining lipid metabolism at a physiological level and preventing the development of hypercholesterolemia are assumed:

deconjugation of bile acids and reduction of their resorption due to the synthesis of specialized hydrolases;

incorporation of cholesterol into the lipid layer of the cell membrane;

transformation of cholesterol into coprostanol and its removal from the body together with feces;

inhibition of cholesterol synthesis in the liver [1-6].

The aim of the study was to establish the cholesterase activity *in vitro* and *in vivo* of previously selected highly probiotic strains of lactic acid bacteria for the further creation on their basis of a line of effective functional foods with hypocholesterolemic activity for the prevention and concomitant treatment of pathological conditions associated with high cholesterol levels.

Materials and methods: The object of the study were strains of lacto- and bifidobacteria isolated from the associative culture during laboratory studies of fermented biological material, deposited in the depository of the Zabolotny Institute of Microbiology and Virology, NAS of Ukraine and patented: *Bifidobacterium bifidum* VK-1, *Bifidobacterium longum* VK-2, *Lactobacillus acidophilus* VK-3 IMV B-7279 (Pat. 93132 UA, publ. 10.01.2011, Bul. 1), *Lactobacillus casei* VK-4 IMV B-7280 (Pat. 93133 UA, publ. 10.01.2011, Bul. 1), *Lactobacillus bulgaricus* VK-5 IMV B-7281 (Pat. 92983 UA, publ. 27.12.2010, Bul. 24). The studies were conducted using bacteria freeze dried in Cuddon Freeze Dryer FD (New Zealand). Before each experimental study, the activity of these freeze-dried probiotic strains was checked by controlling their growth on a Man-Rogosa-Sharpe Agar or Bifido Agar medium (Merck, Germany) at 37°C during 24-48 hours.

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). Bacterial influence on maintenance of

cholesterol concentration in MRS broth was determined according to Rudel L.L. after 18 and 24 hours of cultivation.

For determining cholesterol lowering activity of probiotic bacteria and their compositions *in vivo* in the experiments were used white mice weighing 16-18 and 18-20 g, male mice of the Balb/c aged 2.5 months and female mice Balb/c aged 3 months.

The animals keeping and all manipulations against them was carried out in according with the Law of Ukraine 3447-IV "On the Protection of Animals from Cruel Treatment", "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes of September 20, 1985", "General Ethical Animal Experiments" (First National Congress on Bioethics, 2001) and "Code of Practice for the Housing and Care of Animals Used in Scientific Procedures".

Experimental hypercholesterolemia was simulated in mice by feeding high-calorie diet for a week. Crystalline cholesterol chemical purity of > 99% (Sigma, USA) was added to the diet. This model, designed by us (Pat. 61954 UA, publ. 10.08.2011, Bul. 15), allows raising the serum cholesterol levels in mice by 46.54 ± 2.1% at an average as compared with intact mice.

Two schemes of administration of the probiotic strains – the prophylactic and therapeutic ones were worked out in the study. According to the prophylactic scheme mice of the experimental group received *per os* 0.3 ml of freshly prepared suspensions of the freeze-dried probiotic cultures, their combinations in concentrations of $3x10^8$ cells/ml, and mixed fodder during 4 days. On the fifth day the mice received high-calorie diet and continued to receive probiotic cultures every day until the end of the diet (seven days). On the first, third and seventh day since the beginning of high-calorie diet the level of total serum cholesterol in animals was determined. Cholesterol-lowering activity (cholesterase activity) was calculated by a decrease of concentration of serum cholesterol in mice which received high-calorie diet and probiotic cultures or their combinations in comparison with the control group of mice, which received only high-calorie diet. Cholesterol-lowering activity was evaluated in per cents from the control group of mice.

The therapeutic scheme provided co-administration of high-calorie diet and probiotic cultures in the diet of mice in the same doses as in the prophylactic scheme. The blood samples were also taken from the animals to analyze the level of total cholesterol on the first, third and seventh day of the experiment, respectively.

Two control groups of mice were used: the first (control) group included the intact mice, which diet included only the standard feed, the second one (control + diet) included mice which diet included only high-calorie diet with no addition of probiotic cultures.

Results: In this study the quantitative indexes of *Lactobacillus* and *Bifidobacterium* cholesteraze activity *in vitro* were determined. 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. Thus, among studied strains the ability to bind cholesterol is not unique, although each strain did have a specific level of cholesteraze activity.

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: *L. casei* VK-4 IMV B-7280 > *L. bulgaricus* VK-5 IMV B-7281 > *L. acidophilus* VK-3 IMV B-7279 > *B. longum* VK-2 > *B. bifidum* VK-1.

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

The results of the research suggest that for creation of complex probiotic with cholesteraze activity the most perspective compositions of lacto- and bifidobacteria are: *B.*

longum VK-2 and *B. bifidum* VK-1, *L. casei* VK-4 IMV B-7280 and *B. bifidum* VK-1, *L. casei* VK-4 IMV B-7280 and *B. longum* VK-2, *L. casei* VK-4 IMV B-7280 and *L. bulgaricus* VK-5 IMV B-7281, as well as *L. casei* VK-4 IMV B-7280 and *L. acidophilus* VK-3 IMV B-7279.

According to the literature, the manifestations of probiotic properties *in vitro* and *in vivo* experiments can differ significantly, namely, strains that had high probiotic properties *in vitro* may not show them at all *in vivo* experiments, and on the contrary, strains that did not show positive activities *in vitro* may turn out to be highly active in *in vivo* experiments [1,2,6].

Regardless of breed, age, sex, body weight of mice and administration scheme of probiotic cultures their cholesterase activity increases to the seventh days of observation. It should also be noted that the prophylactic schemes of probiotic cultures administration had higher values of cholesterase activity than therapeutic ones. This suggests that the prevention of disease is the best treatment.

Strains *L. acidophilus* VK-3 IMV B-7279 and *B. bifidum* VK-1, as well as composition *B. bifidum* VK-1 + *B. longum* VK-2 (strains' ratio 1:1) were the most effective probiotics used for treatment of mice with hypercholesterolemia. So, it can be marked that cholesterol-lowering activity of the studied probiotic strains and their compositions ranged between 40-78%.

Conclusions. So far as cholesterol-lowering activity of the studied probiotic strains and their compositions ranged between 40-78%, it should be noted that the cholesterase activity of the other studied strains was not lower, and in some cases even higher than that of most drugs currently used in cholesterinosis: Rosuvastatin, Lovastatin, Fluvastatin, Atorvastatin etc.

Thus, the selected cultures of probiotic lactic acid bacteria and their compositions could potentially be used to create on their basis new effective functional food products and probiotics, as well as dietary supplement, to reduce serum cholesterol in humans. Probiotics that contain cholesterol-assimilating strains of lactic acid bacteria can efficiently complete the complex therapy of patients with cardiovascular, cancer and other diseases.

Such products and drugs are devoid of the negative side effects inherent in statins, especially hepatotoxicity, they are not addictive and do not require lifelong use. Also, functional food products enriched with such probiotic microorganisms with hypocholesterolemic activity can be used not only in therapy, but also in the prevention of diseases associated with high levels of serum cholesterol. And as you know, prevention is the best, health-friendly and effective treatment for any disease.

Literature:

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