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SELECTION OF PROBIOTIC MICROORGANISMS AND THEIR COMPOSITIONS AS A BASIS OF LINE FUNCTIONAL FOOD PRODUCTS WITH HYPOCHOLESTEROLEMIC PROPERTIES

*In modern clinical practice, the main methods for correcting elevated serum cholesterol levels are drugs that block the activity of the enzyme hydroxymethylglutaracyl-CoA-reductase (HMG-CoA-reductase) — statins or drugs that inhibit the absorption of cholesterol and sterols in the intestines ezetimibe. All hypocholesterolemic drugs are rather expensive and have side effects, the main of which is hepatotoxicity. During mono- and combined therapy with HMG-CoA reductase inhibitors and ezetimibe, cases of increased activity of alanine and asparagine transaminases (biochemical indicator of cytolytic syndrome), disorders of the digestive and respiratory systems, the central and peripheral nervous systems, and the sense organs along with weight gain, etc. have been found. Scientific literature has increasingly reported on the ability of lactic acid bacteria to lower serum cholesterol. The ability of certain strains of representatives of the normal microbiota to assimilate and precipitate deconjugated bile acids, as well as to destroy, bind, and assimilate cholesterol, is the basis of their hypocholesterolemic activity (the ability to reduce the level of serum cholesterol). A high level of cholesterol, both in the total blood serum and in low-density lipoproteins, is one of the main risk factors for coronary heart disease, atherosclerosis, cerebrovascular atherosclerosis, hypertension, tumors of the digestive tract, etc. The **aim** of the study was to establish the hypocholesterolemic activity in vitro and in vivo of previously selected highly effective probiotic strains of lactic- and bifidobacteria for the further creation on their basis of a line of effective functional food with hypocholesterolemic activity for the prevention and concomitant treatment of pathological conditions associated with*

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high cholesterol levels. ***Methods****. Bacterial hypocholesterolemic activity in vitro was determined according to Rudel L.L. and in vivo — on the mice model that was designed by us. Two schemes of the administration of probiotic strains — the prophylactic and therapeutic ones—were developed.* ***Results*** *showed that Lactobacillus acidophilus IMV B-7279 and Bifidobacterium animalis IMV B-7286 strains, as well as the Bifidobacterium animalis IMV B-7286: Bifidobacterium animalis IMV B-7285 (1:1) composition were the most effective probiotics used for treatment of mice with hypercholesterolemia. The cholesterol-lowering activity of all studied probiotic strains and their compositions ranged between 40—78%. At the same time, it should be noted that the hypocholesterolemic activity of the other studied strains was not lower, and in some cases even higher than that of most of the drugs currently used for cholesterinosis.* ***Conclusions.*** *The obtained results allow us to assert that it is necessary to develop a series of functional foods and probiotics based on the studied strains and their compositions in an encapsulated form, for the prevention and treatment of diseases associated with the negative manifestations of high cholesterol levels.*

Keywords: *functional food, probiotics, cholesterol-lowering activity, hypocholesterolemic activity, cholesterol.*

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. Obligate microbiota provides hypolipidemic and hypotensive effects by deconjugating bile acid salts, assimilating and precipitating cholesterol, and reducing the activity of tissue angiotensin-converting enzyme (Hassan et al., 2019; Bhat et al., 2019; Daliri et al., 2022; Alaqil et al., 2020; O'Morain & Ramji, 2020; Jia et al., 2023; Asan-Ozusaglam & Gunyakti, 2019; Park et al., 2018; Damodharan et al., 2016; Bendali et al., 2017; Singhal et al., 2019; Zhang et al., 2017; Saikia et al., 2018; Bidura et al., 2019; Majeed et al., 2019; Huang et al., 2021; Haldar & Gandhi, 2019).

The role of the intestinal microbiota in the metabolism of bile acids, which perform an important function in lipid metabolism, is significant. Primary (cholic and chenodeoxycholic acids) and secondary (deoxycholic and lithocholic acids) bile acids are most widely represented in the human body. Each bile acid differs in chemical composition and biological activity.

Bile acids are reabsorbed during active transport in the ileum, enter the liver, are processed again in the liver, and secreted into the duodenum. Conjugated cholic acid, which was not absorbed in the ileum, is deconjugated in the colon by microbial choleglycine hydrolase and dehydroxylated with the participation of 7-α-dehydroxylase to form deoxycholic acid (when pH increases, deoxycholic acid is ionized and well absorbed by the large intestine, and displayed when pH decreases). Absorption of deoxycholic acid ensures replenishment of the pool of bile acids and is an important factor in regulating cholesterol synthesis. An increase in the pH value in the large intestine (may be due to various reasons) leads to an increase in the activity of enzymes that lead to the synthesis of deoxycholic acid, to an increase in its solubility and absorption, and therefore to an increase in the level of bile acids, cholesterol, and triglycerides in the blood (Singhal et al., 2019; Tom et al., 2021).

The participation of the colonic microbiota in cholesterol metabolism is very important. Intestinal microorganisms metabolize cholesterol into coprostanol and further to coprostanol 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 (Hassan et al., 2019; Bhat et al., 2019; Daliri et al., 2022; Alaqil et al., 2020; O'Morain & Ramji, 2020; Jia et al., 2023; Asan-Ozusaglam & Gunyakti, 2019; Park et al., 2018).

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

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

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

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

4) inhibition of cholesterol synthesis in the liver (Hassan et al., 2019; Bhat et al., 2019; Daliri et al., 2022; Alaqil et al., 2020; O'Morain & Ramji, 2020; Jia et al., 2023; Asan-Ozusaglam & Gunyakti, 2019; Park et al., 2018; Damodharan et al., 2016; Bendali et al., 2017; Singhal et al., 2019; Zhang et al., 2017; Saikia et al., 2018; Bidura et al., 2019; Majeed et al., 2019; Huang et al., 2021; Haldar & Gandhi, 2019; Tom et al., 2021; Palaniyandi et al., 2020; Yusuf et al., 2020; Fernandez-Calderon et al., 2022).

Apart from modulating gut functionality, probiotics have also been associated with various other health benefits such as brain functioning, boosting immunity, reducing cholesterol and promoting metabolic homeostasis through their biological mechanisms in the body. «Probiotic Supplements Market — Global Outlook and Forecast 2020—2025» reported that the growth in immune health concerns among people led to an elevation in the market growth of probiotic supplements during the COVID-19 pandemic. «Probiotics Market — Growth, Trends, and Forecast» forecasted the global probiotics market to reach USD 76.85 billion by 2024, registering a compound annual growth rate (CAGR) of 8.15% during the forecast period 2020—2025. The report also indicated that the bacteria market would grow at the fastest CAGR, owing to the growing demand for prominent applications in fortifying foods with probiotics (Yoha et al., 2022).

FAO/WHO 2002 reported that the viability of probiotics in food products must be in adequate amounts to confer a health benefit. Also, it has been reported that probiotic foods should have at least 10^6 CFU/g viability of live microorganisms (Yoha et al., 2022; Global Food 2019—2024). However, the stability of probiotics is the most desirable concern for targeted colon delivery when ingested orally. It is necessary to maintain its viability during gastrointestinal (GI) transit to promote its efficacy. Probiotics require protection against various stress factors during processing, storage, and digestion. Different strains of probiotics show variations in their abilities such as functional properties, stability, and efficacy.

The «Food Encapsulation: Global Market Analysis, Trends, and Forecasts» report has highlighted the encapsulation market outlook (2019—2024) on probiotics' microencapsulation to drive the growth of this sector (Global food, 2019-2024). The recent report «Food encapsulation market by shell material, technology, application, method, core phase, and the region» forecasted the food encapsulation market to reach USD 14.1 billion by 2025, registering a CAGR of 7.5% in 2020—2025, or 8.2% in 2022—2027 (Food encapsulation 2025; Food encapsulation 2027).

Therefore, the **aim** of the study was to establish the hypocholesterolemic activity *in vitro* and *in vivo* of previously selected highly effective probiotic strains of lactic acid bacteria for the further creation on their basis of a line of effective functional encapsulation food with hypocholesterolemic activity for the prevention and concomitant treatment of pathological conditions associated with high cholesterol levels, such as cerebrovascular atherosclerosis, hypertension, tumors of the digestive tract, etc.

Materials and Methods. The objects 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 Zabolotny Institute of Microbiology and Virology, NAS of Ukraine: *Bifidobacterium animalis* IMV B-7286, *Bifidobacterium animalis* IMV B-7285, *Lactobacillus acidophilus* IMV B-7279 (Pat. 93132UA, publ. 10.01.2011, Bul.1), *Lactobacillus casei* IMV B-7280 (Pat. 93133UA, publ. 10.01.2011, Bul.1), *Lactobacillus delbrueckii* subsp. *bulgaricus* IMV B-7281 (Pat. 92983UA, 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 (MRS) Agar for *Lactobacillus* and Bifido Agar for *Bifidobacterium* (Merck, Germany) at 37 °C during 24-48 hours.

To determine the bacterial hypocholesterolemic activity in vitro, freeze-dried cultures of lactobacteria and 48-hour cultures of bifidobacteria were used, inoculated in MRS broth supplemented with sodium thioglycollate (Sigma), Oxgall (Difco Laboratories) and freshly (extempore, newly) prepared cholesterol (chemical purity > 99%, Sigma-Aldrich, USA). Bacterial influence on the maintenance of cholesterol concentration in MRS broth was determined according to Rudel L.L. after 18 and 24 hours of cultivation.

Table 1. **Composition of the diet based on corn meal**

Components	Quantity, g
Corn meal	135.5
Butter	27.2
Wheat bran	72.6
Soybean meal	98.0
NaCl	0.9
$CaHPO_4$	5.4
$CaCO_3$	3.3
Vitamins, macro- and microelements*	1.5

*Comments: The composition of diet consists of the following vitamins, macro- and microelements: riboflavin — 1.76 µg; pantothenic acid — 8.80 µg; niacin — 8.80 µg; vitamin B_{12} — 8.80 µg; choline chloride — 176.00 µg; vitamin A — 1760 lU; vitamin D_3 — 176 lU; vitamin E — 4.4 lU; and also complex of macro- and microelements: selenium — 39.6 µg, iodine — 300 µg; iron — 19.8 µg; manganese — 11 µg, copper — 2.2 µg, zinc — 39.6 µg per 1 kg of feed.

To determine cholesterol-lowering activity of probiotic bacteria *in vivo,* we used male white BALB/c line mice weighing 16—18 and 18—20 g, aged 2.5 months and female white BALB/c mice aged 3 months.

The animals were kept in plastic cages under standard vivarium conditions in a separate room (air temperature 20—22 °C). The keeping of animals and all manipulations was carried out by 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» (Strasbourg, September 20, 1986), «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» (Code of Practice).

Experimental hypercholesterolemia was simulated in mice by feeding them with a high-calorie diet (Table 1) for a week. Crystalline cholesterol with chemical purity > 99% (Sigma, USA) was added to the diet. This model, designed by us (Pat. 61954UA, 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 probiotic strain administration, namely the prophylactic and therapeutic ones, were used 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 or their combinations in the concentrations of 3 x 10^8 cells/mL (in physiological solution) for 4 days. During this period, mice had free axes to mixed fodder. Starting from the 5^{th} day, mice started to receive a 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 days after the beginning of the high-calorie diet, the level of total serum cholesterol in animals was determined. Cholesterol-lowering activity (hypocholesterolemic activity) was calculated by a decrease in the concentration of serum cholesterol in mice that received the high-calorie diet and probiotic cultures or their combinations in comparison with the control group of mice that received only the high-calorie diet. Cholesterol-lowering activity was evaluated in percent from the control group of mice.

The therapeutic scheme provided co-administration of the 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 days of the experiment.

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

All experiments were performed at least three times using appropriate controls. The digital data obtained were processed by statistical methods using Student's t-test. The difference was considered reliable when $p < 0.05$.

Results. Considering the established requirements for probiotic microorganisms, all strains of lactic acid bacteria were previously tested for compliance with these requirements (Binda et al., 2020; Palanivelu et al., 2022).

An important requirement for any probiotic microorganism is the ability to survive under the aggressive conditions of the gastrointestinal tract (GIT). It was experimentally proven that the selected probiotic strains are resistant to:

- gastric juice in a wide range of concentrations (1, 2, 5, 8, 10, 20, 30, 50, 75, 100%) during 2.5 hours of observation. It should be noted that even up to 100% concentration of gastric juice, *B. animalis* IMV B-7286, *B. animalis* IMV B-7285, and *L. acidophilus* IMV B-7279 strains were resistant. *L. casei* IMV B-7280 showed resistance to a 50% concentration of gastric juice. The growth of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 was completely inhibited by the 10% concentration of gastric juice;
- bile (0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 75%) for 5 hours of observation. On a positive note, all tested strains partially retained their viability even under bile concentrations of 40%;
- proteolytic enzymes (0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5%) for 5 hours of observation;
- complex effect of aggressive factors of the GIT (gastric juice 2%, bile acids 1%, and proteolytic enzymes 1%). Even under the complex influence of aggressive conditions on cells, the survival of *B. animalis* IMV B-7286, *B. animalis* IMV B-7285, *L. acidophilus* IMV B-7279, and *L. casei* IMV B-7280 was 90%, only for *L. casei* IMV B-7280 survival was 79.2%, which was also a good result (Bubnov et al, 2023).

One of the most important criteria assessing the prospects of strains for creating probiotics or probiotic-based functional food is the ability of these bacteria to suppress the growth and reproduction of pathogenic and opportunistic microorganisms. The antagonistic activity of probiotic microbiota is one of the mechanisms of ensuring the colonization resistance of the macroorganism. The antagonism of lacto- and bifidobacteria to the host's pathogenic and opportunistic microbiota is realized by the synthesis of organic acids, hydrogen peroxide, lysozyme, antibiotics, bacteriocins, competition for nutrients, high rate of reproduction of cell populations, etc.

The results of the research showed that all strains of lacto- and bifidobacteria inhibited the growth of the test cultures used in the experiment.

The greatest antagonistic activity to either museum or clinical strains of pathogenic and opportunistic microorganisms, including yeast genus *Candida*, was exhibited by *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, and *B. animalis* IMV B-7286, although other strains also had high values of zones of growth retardation relative to all pathogenic microorganisms used. It is noteworthy that the growth of some test cultures was almost completely inhibited by certain strains of lacto- and bifidobacteria.

With the development of the latest technologies, one of the popular directions is creating complex probiotics and/or functional food containing several different strains, species, and genera of microorganisms. This direction becomes promising because, complementing each other, these bacteria have a greater spectrum and effectiveness of therapeutic action compared to monoprobiotics.

Determination of the biocompatibility of bacteria showed that all five strains of lactic acid bacteria used are not antagonists to each other. The obtained data open the possibility of combining the studied strains of lacto- and bifidobacteria in different combinations and with different volume ratios, thereby obtaining functional food and probiotics with different probiotic properties for the prevention and therapy of patients with different microbiota imbalances and related accompanying pathologies.

The adhesive properties of probiotic microorganisms deserve special attention. Every microorganism that enters the digestive tract from the outside or is an obligatory component of the microbiocenosis tries to attach itself to the intestinal epithelium. This enables it to occupy the most convenient ecological niche, able to provide the microbial cell with readily available substrates, protect it from competition with other microorganisms, and prevent the elimination from intestines during peristalsis. Adhesive strains of microorganisms play a major role in probiotic therapy. All studied strains were classified as highly adhesive and arranged in a row according to the decrease in their adhesive properties: *L. casei* IMV B-7280 > *B. animalis* IMV B-7286 > *B. animalis* IMV B-7285 > *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 > *L. acidophilus* IMV B-7279.

Considering that most probiotics are used during and/or after antibiotic therapy, it was reasonable to check the resistance of the studied probiotic strains to modern chemotherapeutic drugs. All strains showed resistance to oxacillin but were sensitive to other penicillins, and were also slightly sensitive and resistant to the cephalosporins of the I, II, III, and IV generations (whereas the *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 strain was resistant to cephalexin, cefuroxime, ceftazidime, ceftibuten, and cefepime). All strains were resistant to glycopeptides (teicoplanin, vancomycin), but showed slight sensitivity to carbapenems. The strains were poorly sensitive to aminoglycosides of the I, II, and III generations, sensitive to macrolides of the I and II generations (except *B. animalis* IMV B-7285, which showed resistance to azithromycin, and *B. animalis* IMV B-7286, which showed resistance to clarithromycin). All strains were sensitive to lincosamides and tetracyclines (except *B. animalis* IMV B-7286, which showed resistance to chlortetracycline). *B. animalis* IMV B-7285 and *L. casei* IMV B-7280 showed resistance to the first generation of nitrofurans, but all strains were insensitive to the II generation of nitrofurans. It should be noted that all strains showed resistance to fluoroquinolones of the I, II, and III generations (except sparfloxacin, to which all strains were sensitive, except *L. casei* IMV B-7280, which was resistant) (Bubnov et al, 2023).

Therefore, all studied strains comply with the requirements for probiotic strains and can be recommended as the basis of probiotic or functional food.

Since the main goal of the work was to develop a line of effective functional food enriched with probiotic strains and probiotic combinations with a significant hypocholesterolemic effect, the strains were further investigated for their ability to lower cholesterol levels first *in vitro* and then *in vivo*.

In this study, the quantitative indexes of *Lactobacillus* and *Bifidobacterium* hypocholesterolemic activity *in vitro* were determined. It was demonstrated that all studied strains of lacto- and bifidobacteria are able to decrease the level of cholesterol in MRS broth both after 18- and

24-hour cultivation. In the case of 18-hour cultivation, the maximal hypocholesterolemic activity was shown by *L. casei* IMV B-7280 — 34.40 ± 0.28%. For *L. acidophilus* IMV B-7279 and *L. delbrueckii subsp. bulgaricus* IMV B-7281 strains, the hypocholesterolemic activity was 24.73 ± 1.22 and 22.05 ± 0.98%, respectively. For *Bifidobacterium* strains, the value of hypocholesterolemic activity was the following: *B. animalis* IMV B-7285 — 17.78 ± 1.21% decrease; *B. animalis* IMV B-7286 — 5.38 ± 0.22% decrease.

24-hour cultivation led to an even more significant reduction of cholesterol concentration in the MRS broth. The maximal hypocholesterolemic activity was demonstrated by *L. casei* IMV B-7280 strain — 62.37 ± 1.68% decrease in the cholesterol level. The same parame ter in the case of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 and *L. acidophilus* IMV B-7279 decreased by 38.71 ± 1.21 and 27.96 ± 1.10% respectively. The amount of eaten up (bound and degraded) cholesterol in the MRS broth by *B. animalis* IMV B-7285 and *B. animalis* IMV B-7286 after 24 hours was 22.46 ± 0.80 and 7.64 ± 0.32%.

It should be noted that the hypocholesterolemic activity of lactic acid bacteria was higher than that of bifidobacteria: *L. casei* IMV B-7280 > *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 > *L. acidophilus* IMV B-7279 > *B. animalis* IMV B-7285 > *B. animalis* IMV B-7286.

It was experimentally established by many works (Daliri et al., 2022; Alaqil et al., Asan-Ozusaglam & Gunyakti, 2019; Park et al., 2018; Damodharan et al., 2016; Bendali et al., 2017; Singhal et al., 2019; Zhang et al., 2017; Palaniyandi et al., 2020; Yusuf et al., 2020; Fernandez-Calderon et al., 2022) that the different strains of lacto- and bifidobacteria are able to increase their beneficial properties if applied in combination with other probiotics. Thus, the determination of hypocholesterolemic activity of different bacteria compositions was the next stage of the experiment. The obtained data are presented in Table 2.

Among all studied compositions (Table 2), only one — *L. bulgaricus* IMV B-7281 : *B. animalis* IMV B-7286 (1:1) did not show choesteraze activity under the conditions of this experiment. The composition consisting of all five studied lacto- and bifidobacteria strains had the average value of hypocholesterolemic activity. For the compositions *L. acidophilus* IMV B-7279 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1), *L. acidophilus* IMV B-7279 : *B. animalis* IMV B-7285 (1:1), *L. acidophilus* IMV B-7279 : *B. animalis* IMV B-7286 (1:1), and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 : *B. animalis* IMV B-7285 (1:1), hypocholesterolemic activity was lower than the level of cholesterol absorption after 18 hours. Its range was 12.0—20.15%, and after 24 hours — 16.16—26.78%. The compositions of *L. casei* IMV B-7280 : *B. animalis* IMV B-7286 (1:1), *L. casei* IMV B-7280 : *B. animalis* IMV B-7285 (1:1), *L. casei* IMV B-7280 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1), and also *L. acidophilus* IMV B-7279 : *L. casei* IMV B-7280 (1:1) demonstrated the highest levels of hypocholesterolemic activity. It should be noted that the combination of *L. acidophilus* IMV B-7279 : *L. casei* IMV B-7280 (1:1) both after 18- and 24-hour cultivation posessed hypocholesterolemic activity at a similar level with that of *L. casei* IMV B-7280 strain alone. Each strain of lactic acid bacteria, constituting the composition *L. casei* IMV B-7280 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1), showed the highest hypocholesterolemic activity compared to the separate cultivation. The substantial increase in cholinesterase activity for the composition *B. animalis* IMV B-7285 : *B. animalis* IMV B-7286 (1:1) was quite unexpected. It is noteworthy that for this composition, hypocholesterolemic activity after 18- and 24-hour cultivations exceeded by 2—10 times the corresponding values of the separate strains. It can be assumed that the studied strains of bifidobacteria in the 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 studied strains of lacto- and bifidobacterial, the highest hypocholesterolemic activity *in vitro* was demonstrated by *L. casei* IMV B-7280. Rather high hypocholesterolemic activity *in vitro* characterized the bacteria composition based on *L. casei* IMV B-7280 and one of the other studied lacto- or bifidobacteria strains. And only for one combination *L. acidophilus* IMV B-7279 : *L. casei* IMV B-7280 (1:1), joint cultivation did not lead to a higher hypocholesterolemic activity compared to the corresponding parameter of separately cultivated *L. casei* IMV B-7280. The strains *B. animalis* IMV B-7285 and *B. animalis* IMV B-7286 are individually characterized as low-cholesterol binders *in vitro*. At the same time, the joint cultivation of these bacteria led to a substantial increase in their hypocholesterolemic effectiveness.

The results of the research suggest that for creation of complex probiotic preparations with hypocholesterolemic activity, the most perspective compositions of lacto- and bifidobacteria are *B. animalis* IMV B-7285 : *B. animalis* IMV B-7286 (1:1), *L. casei* IMV B-7280 : *B. animalis* IMV B-7286 (1:1), *L. casei* IMV B-7280 : *B. animalis* IMV B-7285 (1:1), *L. casei* IMV B-7280 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1), and *L. casei* IMV B-7280 : *L. acidophilus* IMV B-7279 (1:1).

Table 2. **Hypocholesterolemic activity of lacto- and bifidobacteria compositions *in vitro***

Composition	Strains' ratio	Cultivation time, h	Cholesterol reduction in MRS broth, %
L. casei IMV B-7280 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 : *L. acidophilus* IMV B-7279 : *B. animalis* IMV B-7285 : *B. animalis* IMV B-7286	1:1:1:1:1	18	28.17 ± 0.01*
		24	38.03 ± 0.02*
L. casei IMV B-7280 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281	1:1	18	33.80 ± 0.03
		24	73.24 ± 0.37
B. animalis IMV B-7285 : *B. animalis* IMV B-7286	1:1	18	54.23 ± 0.28*
		24	68.87 ± 0.36*
L. casei IMV B-7280 : *B. animalis* IMV B-7286	1:1	18	49.30 ± 0.21*
		24	52.82 ± 0.23*
L. delbrueckii subsp. *bulgaricus* IMV B-7281 : *B. animalis* IMV B-7285	1:1	18	14.79 ± 0.21*
		24	23.15 ± 0.03*
L. casei IMV B-7280 : *B. animalis* IMV B-7285	1:1	18	43.94 ± 0.08*
		24	57.75 ± 0.18*
L. acidophilus IMV B-7279 : *L. casei* IMV B-7280	1:1	18	32.88 ± 0.19*
		24	64.92 ± 0.07*
L. acidophilus IMV B-7279 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281	1:1	18	20.15 ± 0.11*
		24	26.78 ± 0.26*
L. acidophilus IMV B-7279 : *B. animalis* IMV B-7285	1:1	18	18.92 ± 0.25*
		24	20.11 ± 0.15*
L. acidophilus IMV B-7279 : *B. animalis* IMV B-7286	1:1	18	12.07 ± 0.08*
		24	16.16 ± 0.22*
p	1:1	18	0
		24	0

*$p < 0.05$ in relation to the composition of *L. casei* IMV B-7280: *L. delbrueckii* subsp. *bulgaricus* IMV B-7281.

The manifestations of probiotic properties in *in vitro* and *in vivo* experiments can significantly differ, for example, 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 vivo* experiments.

The next stage of the experimental work was the determination of the hypocholesterolemic activity of bacteria of *Lactobacillus* and *Bifidobacterium* genera in the experimental model of hypercholesterolemia in mice. The results are shown in Tables 3 and 4.

The data presented in Table 3 show that on the first day of using the therapeutic scheme of probiotic administration in mice weighing 18—20 g, the maximum of hypocholesterolemic activity was observed for *L. acidophilus* IMV B-7279 — 31.15 ± 1.4%, while the minimum was observed for *L. acidophilus* IMV B-7279 + *L. casei* IMV B-7280 (1:1) — 3.0 ± 0.1%, and *L. casei* IMV B-7280 + *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1) — 4.32 ± 0.2%. For the other cultures, hypocholesterolemic activity ranged from 8.44 to 16.07% on the 3rd day of observation, the maximum hypocholesterolemic activity remained at 37.11 ± 1.6% for *L. acidophilus* IMV B-7279, and the minimum value was 17.84 ± 0.7% for *L. casei* IMV B-7280. The maximum hypocholesterolemic activity on the 7th day of observation was shown for *L. casei* IMV B-7280 strain — 62.28 ± 2.5% and minimum — for the composition *L. acidophilus* IMV B-7279 + *L. casei* IMV B-7280 (1:1) — 28.70 ± 1.2%. For the other strains, hypocholesterolemic activity was almost the same and varied within 43.01 ± 1.7%.

Hypocholesterolemic activity for mice weighing 16—18 g under the administration of probiotic bacteria by the therapeutic scheme (Table 3): the maximum values of hypocholesterolemic activity were shown by the compositions *L. acidophilus* IMV B-7279 + *L. casei* IMV B-7280 (1:1) — 43.38 ± 1.5% and *B. animalis* IMV B-7286 + *B. animalis* IMV B-7285 (1:1) — 64.78 ± 2.7% on the 1st, 3rd, and 7th days, respectively. On the first day, the minimum values were 16.66 ± 0.7% for *L. casei* IMV B-7280 and 16.63 ± 0.6% for *L. casei* IMV B-7280 + *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1), on the third and seventh day they were 35.19 ± 1.3 and 48.16 ± 1.8%, respectively, for the composition *L. casei* IMV B-7280 + *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1). On the seventh day of observation, the average value of hypocholesterolemic activity was 33.0 ± 2.3%, which is 13.32 ± 0.5% higher than for mice weighing 18—20 g. The obtained data suggest that it is easier to restore the organisms of young mice under the therapeutic administration of probiotic cultures than the organisms of more mature mice.

Hypocholesterolemic activity of probiotic cultures in male BALB/c mice aged 2.5 months in prophylactic and therapeutic schemes of probiotic cultures administration showed the following results (Tables 3 and 4).

For the prophylactic scheme of probiotic cultures administration (Table 4), the hypocholesterolemic activity for all strains and their compositions were characterized by practically the same values and amounted to 33.63 ± 1.4% on the 1st day, 45.41 ± 1.6% on the 3rd day, and 65.29 ± 2.6% on the 7th day, respectively. The maximum value of hypocholesterolemic activity on the 7th day was observed for *L. acidophilus* IMV B-7279 — 69.58 ± 2.8%. *L. acidophilus* IMV B-7279 and the composition of *B. animalis* IMV B-7286 + *B. animalis* IMV B-7285 (1:1) were the most effective in the prophylactic scheme. The same trend remained for the therapeutic scheme. On the first day, the average hypocholesterolemic activity ranged from 8.68% for *L. casei* IMV B-7280 to 19.87—20.98% for *L. acidophilus* IMV B-7279 and compositions *B. animalis* IMV B-7286 + *B. animalis* IMV B-7285 (1:1) and *L. casei* IMV B-7280 + *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1). The average value of hypocholesterolemic activity on the 3rd day of the therapeutic scheme was 35.11 ± 1.4%. On the 7th day, hypocholesterolemic activity increased the minimum to 56.5% for *L. casei* IMV B-7280 and composition *L. casei* IMV B-7280 + *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1) and the maximum — to 65.84 ± 2.6% for the composition *B. animalis* IMV B-7286 + *B. animalis* IMV B-7285 (1:1).

Hypocholesterolemic activity of female BALB/c mice aged 3 months when using the pro-

Table 3. **Hypocholesterolemic activity of lacto- and bifidobacteria compositions *in vivo* under the therapeutic scheme of probiotics' administration**

Probiotic strain or composition (strains' ratio)	Hypocholesterolemic activity, %		
	On the 1st day of investigation	On the 3rd day of investigation	On the 7th day of investigation
mice weighing 18—20 g			
L. acidophilus IMV B-7279	31.15 ± 1.4%	37.11 ± 1.6%	44.7 ± 2.0%
L. casei IMV B-7280	11.22 ± 0.5%	17.84 ± 0.7%	62.28 ± 2.5%
L. acidophilus IMV B-7279 : *L. casei* IMV B-7280 (1:1)	3.0 ± 0.1%	21.90 ± 0.9%	28.70 ± 1.2%
B. animalis IMV B-7286	8.44 ± 0.3%	31.53 ± 1.4%	46.56 ± 2.1%
B. animalis IMV B-7285	9.39 ± 0.4%	26.76 ± 1.2%	44.59 ± 2.0%
B. animalis IMV B-7286 : *B. animalis* IMV B-7285 (1:1)	16.07 ± 0.7%	25.98 ± 1.1%	34.17 ± 1.5%
L. casei IMV B-7280 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281(1:1)	4.32 ± 0.2%	35.91 ± 1.6%	45.03 ± 2.0%
mice weighing 16—18 g			
L. acidophilus IMV B-7279	27.92 ± 1.2%	42.97 ± 1.9%	55.54 ± 2.4%
L. casei IMV B-7280	16.66 ± 0.7%	38.51 ± 1.7%	52.49 ± 2.3%
L. acidophilus IMV B-7279 : *L. casei* IMV B-7280 (1:1)	21.66 ± 0.9%	43.38 ± 1.5%	56.70 ± 2.5%
B. animalis IMV B-7286	18.96 ± 0.8%	41.51 ± 1.8%	57.08 ± 2.5%
B. animalis IMV B-7285	20.70 ± 0.9%	39.89 ± 1.7%	59.53 ± 2.6%
B. animalis IMV B-7286 : *B. animalis* IMV B-7285 (1:1)	22.65 ± 1.0%	37.46 ± 1.6%	64.78 ± 2.7%
L. casei IMV B-7280 : *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1)	16.63 ± 0.6%	35.19 ± 1.3%	48.16 ± 1.8%
male BALB/c mice aged 2.5 months			
L. acidophilus IMV B-7279	19.87 ± 0.8%	34.30 ± 1.5%	64.10 ± 2.8%
L. casei IMV B-7280	8.68 ± 0.4%	27.49 ± 1.2%	56.37 ± 2.5%
L. acidophilus IMV B-7279 : *L. casei* IMV B-7280 (1:1)	11.61 ± 0.5%	33.57 ± 1.5%	59.34 ± 2.6%
B. animalis IMV B-7286	10.96 ± 0.4%	35.85 ± 1.6%	61.06 ± 2.7%
B. animalis IMV B-7285	13.33 ± 0.5%	34.28 ± 1.5%	57.73 ± 2.5%
B. animalis IMV B-7286 : *B. animalis* IMV B-7285 (1:1)	19.85 ± 0.8%	39.72 ± 1.7%	65.84 ± 2.6%
L. casei IMV B-7280: *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1)	20.98 ± 0.9%	40.54 ± 1.8%	56.92 ± 2.5%
female BALB/c mice aged 3 months			
L. acidophilus IMV B-7279	32.01 ± 1.4%	41.97 ± 1.8%	69.59 ± 2.8%
L. casei IMV B-7280	22.12 ± 0.9%	36.98 ± 1.6%	53.89 ± 2.4%
L. acidophilus IMV B-7279: *L. casei* IMV B-7280 (1:1)	28.61 ± 1.2%	38.64 ± 1.7%	58.60 ± 2.6%
B. animalis IMV B-7286	30.06 ± 1.3%	41.81 ± 1.8%	60.03 ± 2.7%
B. animalis IMV B-7285	20.63 ± 0.9%	36.78 ± 1.6%	57.89 ± 2.6%
B. animalis IMV B-7286 : *B. animalis* IMV B-7285 (1:1)	32.49 ± 1.4%	40.42 ± 1.8%	59.98 ± 2.7%
L. casei IMV B-7280: *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1)	20.52 ± 0.9%	33.62 ± 1.5%	51.75 ± 2.3%

phylactic (Table 4) and therapeutic (Table 3) schemes of probiotic bacteria administration was determined as well. In this case, the prophylactic scheme also showed higher values of hypocholesterolemic activity than the therapeutic one. For prophylactic scheme of administration, the hypocholesterolemic activity amounted from 37.01 ± 1.4% on the 1st day, to 57.11 ± 2.3% on the 3rd day, to 68.37 ± 3.0% on the 7th day. The maximum of hypocholesterolemic activity was observed on the 7th day in the group of *L. acidophilus* IMV B-7279 — 78.04 ± 3.0% and composition *B. animalis* IMV B-7286 + *B. animalis* IMV B-7285 (1:1) — 74.08 ± 3.0%.

The therapeutic scheme (Table 3) was characterized by slightly lower average values of hypocholesterolemic activity: 26.63 ± 1.1% for the 1st day, 38.57 ± 1.5% for the 3rd day, and 58.82 ± 2.4% for the 7th day. The maximum value of activity was found for *L. acidophilus* IMV B-7279 — 69.59 ± 2.8%, for the other cultures it varied within 51.75—60.03% on the 7th day of observation.

Recent research in this field conformed completely to the experimental data (Daliri et al., 2022; Alaqil et al., Asan-Ozusaglam & Gunyakti, 2019; Park et al., 2018; Damodharan et al., 2016; Bendali et al., 2017; Singhal et al., 2019; Zhang et al., 2017; Palaniyandi et al., 2020; Yusuf et al., 2020; Fernandez-Calderon et al., 2022).

As seen from Tables 3 and 4, regardless of the breed, age, sex, body weight of mice and administration scheme of probiotic cultures, their hypocholesterolemic activity increases to the 7th days of observation. The prophylactic schemes of probiotic cultures administration had higher values of hypocholesterolemic activity than therapeutic ones. This suggests that the prevention of disease is the best treatment.

Strains *L. acidophilus* IMV B-7279 and *B. animalis* IMV B-7286, as well as the composition *B.*

Table 4. **Hypocholesterolemic activity of lacto- and bifidobacteria compositions *in vivo* under the prophylactic scheme of probiotics' administration**

Probiotic strain or composition (strains' ratio)	Hypocholesterolemic activity, %		
	On the 1st day of investigation	On the 3rd day of investigation	On the 7th day of investigation
male BALB/c mice aged 2.5 months			
L. acidophilus IMV B-7279	37.40 ± 1.6%	48.67 ± 2.1%	69.58 ± 2.8%
L. casei IMV B-7280	28.65 ± 1.2%	38.28 ± 1.7%	63.02 ± 2.8%
L. acidophilus IMV B-7279 : *L. casei* IMV B-7280 (1:1)	30.94 ± 1.3%	42.47 ± 1.9%	65.54 ± 2.9%
B. animalis IMV B-7286	30.44 ± 1.3%	45.63 ± 2.0%	67.00 ± 3.0%
B. animalis IMV B-7285	32.29 ± 1.4%	44.30 ± 1.9%	64.17 ± 2.8%
B. animalis IMV B-7286 : *B. animalis* IMV B-7285 (1:1)	37.38 ± 1.6%	48.92 ± 2.1%	67.61 ± 3.0%
L. casei IMV B-7280: *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1)	38.26 ± 1.7%	49.61 ± 2.2%	60.11 ± 2.7%
female mice BALB/c aged 3 months			
L. acidophilus IMV B-7279	41.88 ± 1.8%	65.69 ± 2.9%	78.04 ± 3.5%
L. casei IMV B-7280	29.32 ± 1.3%	51.94 ± 2.3%	64.46 ± 2.8%
L. acidophilus IMV B-7279 : *L. casei* IMV B-7280 (1:1)	39.54 ± 1.7%	61.13 ± 2.7%	66.04 ± 2.9%
B. animalis IMV B-7286	33.02 ± 1.4%	55.31 ± 2.4%	68.71 ± 3.0%
B. animalis IMV B-7285	36.57 ± 1.6%	56.43 ± 2.5%	67.68 ± 3.0%
B. animalis IMV B-7286 : *B. animalis* IMV B-7285 (1:1)	34.55 ± 1.5%	56.67 ± 2.5%	74.08 ± 3.0%
L. casei IMV B-7280: *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (1:1)	44.16 ± 1.9%	52.66 ± 2.3%	59.58 ± 2.6%

animalis IMV B-7286 + *B. animalis* IMV B-7285 (1:1) were the most effective probiotics used for treatment of mice with hypercholesterolemia. So, it can be marked as the best probiotic strains and their compositions with cholesterol-lowering activity ranged between 40—78%. At the same time, it should be noted that the hypocholesterolemic activity of the other studied strains was not lower, and in some cases was even higher than that of most of the drugs currently used in cholesterolosis.

It is noteworthy that on the basis of one of the investigated strains — *L. casei* IMV B-7280, a dietary supplement Dialak (TU U 10.08096051-004.2015) has already been developed. Dialak is a probiotic (immunobiotic) made from live lactobacilli with immunomodulatory, antimicrobial, and hypocholesterolemic effects.

The obtained results allow us to assert that it is necessary to develop a series of functional foods and probiotics based on the studied strains and their compositions in an encapsulated form (which will increase the survival of probiotic microorganisms passing through the upper gastrointestinal tract, which will indirectly increase the therapeutic effect of such drugs and products) (Starovoitova et al., 2022) for the prevention and treatment of diseases associated with the negative manifestations of high cholesterol levels.

2500 years ago, Hippocrates made a very important statement: «Let food be your medicine and medicine be your food». This concept is used to emphasize the role of bioactive compounds in foods in the prevention or treatment of some chronic diseases (El-Sohaimy et al., 2023).

The field of functional probiotic foods requires the cooperation of food technologists, nutritionists, medical doctors, and food chemists to obtain innovative products. In this way, these foods may be able to adjust physiological parameters related to the health status or disease prevention in humans. So, the design and development of functional probiotic foods is a scientific work as an an expensive and multistage process that takes into account many factors, such as sensory acceptance, physical and microbial stability, price, and chemical and other intrinsic functional properties to be successful on the market (Damián et al., 2022; Plessas et al., 2022).

Conclusions. The cholesterol-lowering activity of the studied probiotic strains and their compositions ranged between 40—78%. At the same time, it should be noted that the cholesterol-lowering activity of the other studied strains was not lower, and in some cases even higher than that of most of the drugs currently used in cholesterolosis (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 and probiotics to reduce serum cholesterol in humans. Probiotics that contain cholesterol-assimilating strains can efficiently complete the complex therapy of patients with cardiovascular diseases, cancer, etc. Such 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 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. As known, prevention is the best, health-friendly, and effective treatment for any disease.

Subsequent studies are planned to increase the percentage of hypocholesterolemic activity by more detailed working out of administration schemes and doses of probiotic cultures, as well as the selection of probiotic strains' combinations and their ratios in these combinations and to design a line of functional food products enriched with the probiotic strains to reduce serum cholesterol levels, and thereby to prevent and treat diseases such as cardiovascular, cancer, and many others.

REFERENCES

Alaqil, A. A., Abbas, A. O., El-Beltagi, H. S., El-Atty, H. K. A., Mehaisen, G. M., & Moustafa, E. S. (2020). Dietary supplementation of probiotic *Lactobacillus acidophilus* modulates cholesterol levels, immune response, and productive performance of laying hens. *Animals*, *10*(9), 1588. https://doi.org/10.3390/ani10091588

Asan-Ozusaglam, M., & Gunyakti, A. (2019). *Lactobacillus fermentum* strains from human breast milk with probiotic properties and cholesterol-lowering effects. *Food Science and Biotechnology*, *28*(2), 501—509. https://doi.org/10.1007/s10068-018-0494-y

Bendali, F., Kerdouche, K., Hamma-Faradji, S., & Drider, D. (2017). *In vitro* and *in vivo* cholesterol lowering ability of *Lactobacillus pentosus* KF923750. *Beneficial Microbes*, *8*(2), 271—280. https://doi.org/10.3920/BM2016.0121

Bhat, B., & Bajaj, B. K. (2019). Hypocholesterolemic potential of probiotics: Concept and mechanistic insights. *Indian Journal of Experimental Biology*, *57*(2), 86—95.

Bidura, I. G. N. G., Siti, N.W., & Partama, I. B. G. (2019). Effect of probiotics, *Saccharomyces* spp. Kb-5 and Kb-8, in diets on growth performance and cholesterol levels in ducks. *South African Journal of Animal Science*, *49*(2), 219—226. http://dx.doi.org/10.4314/sajas.v49i2.2

Binda, S., Hill, C., Johansen, E., Obis, D., Pot, B., Sanders, M. E., Tremblay, A., & Ouwehand, A. C. (2020). Criteria to Qualify Microorganisms as «Probiotic» in Foods and Dietary Supplements. *Front Microbiol*, *11*, 1662. https://doi.org/10.3389/fmicb.2020.01662

Bubnov, R. V., Babenko, L. P., Lazarenko, L. M., Mokrozub, V. V., & Spival, M.Ya. (2023). Chapter 13 *In vitro* study of specific properties of probiotic strains for effective and personalized probiotic therapy. In: Microbiome in 3P medicine strategies. The first exploitation guide. Editors N. Boyko, O. Golubnichaja. 355—370. https://doi.org/10.1007/978-3-031-19564-8_13

Code of Practice for the Housing and Care of Animals Used in Scientific Procedures. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388293/CodePractice-HousingCareAnimals.pdf

Daliri, E. B. M., Kim, Y., Do, Y., Chelliah, R., & Oh, D. H. (2022). *In vitro* and *in vivo* cholesterol reducing ability and safety of probiotic candidates isolated from Korean fermented soya beans. *Probiotics and Antimicrobial Proteins*, *14*(1), 87—98. https://doi.org/10.1007/s12602-021-09798-0

Damián, M. R., Cortes-Perez, N. G., Quintana, E.T., Ortiz-Moreno, A., Garfias Noguez, C., Cruceño-Casarrubias, C. E., Sánchez Pardo, M. E. (2022). Functional Dairy Probiotic Food Development: Trends, Concepts, and Products. *Microorganisms*, *10*(5), 1065. https://doi.org/10.3390/microorganisms10051065

Damodharan, K., Palaniyandi, S. A., Yang, S. H., & Suh, J. W. (2016). Functional Probiotic Characterization and *In Vivo* Cholesterol-Lowering Activity of *Lactobacillus helveticus* Isolated from Fermented Cow Milk. *Journal of Microbiology and Biotechnology*, *26*(10), 1675—1686. https://doi.org/10.4014/jmb.1603.03005

El-Sohaimy, S. A., & Hussain, M. A. (2023). Functional Probiotic Foods Development: Trends, Concepts, and Products. *Fermentation*, *9*(3), 249. https://doi.org/10.3390/fermentation9030249

Fernandez-Calderon, M. C., Sanchez-Moro, M. D. H., & Rincon, E. O. (2022). *In vitro* Cholesterol Assimilation by *Bifidobacterium animalis subsp. lactis* (BPL1) Probiotic Bacteria Under Intestinal Conditions. *Endocr Metab Immune Disord Drug Targets*, *22*(4), 433—439. https://doi.org/10.2174/1871530321666210908124848

Food encapsulation market by shell material, technology, application, method, core phase and region-Global forecast to 2025. https://www.reportlinker.com/p03984708/Food-Encapsulation-Market-by-Shell-Material-Core-Phase-Technology-by-Region-Global-Forecasts-to.html?utm_source=PRN

Food encapsulation market by shell material, technology, application, method, core phase and region-Global forecast to 2027. https://www.reportlinker.com/p03984708/Food-Encapsulation-Market-by-Shell-Material-Core-Phase-Technology-by-Region-Global-Forecasts-to.html?utm_source=PRN

Global food encapsulation market outlook 2019—2024 — Microencapsulation of probiotics to drive growth - ResearchAndMar-kets.com. Business Wire. https://www.businesswire.com/news/ home/20190613005360/en/Global-Food-Encapsulation-Market-Outlook-2019-2024

Haldar, L., & Gandhi, D. N. (2019). Cholesterol-lowering effects of *Bacillus coagulans* B37 and *Bacillus pumilus* B9 strains in a rat animal model. *Indian Journal of Animal Research*, *53*(4), 469—475. https://doi.org/10.18805/ijar.B-3549

Hassan, A., Din, A. U., Zhu, Y., Zhang, K., Li, T., Wang, Y., Luo, Y. & Wang, G. (2019). Updates in understanding the hypocholesterolemia effect of probiotics on atherosclerosis. *Applied microbiology and biotechnology*, *103*(15), 5993—6006. https://doi.org/10.1007/s00253-019-09927-4

Huang, J., Xiao, N., Sun, Y., Wu, S., Tian, W., Lai, Y., Li, P., & Du B. (2021). Supplementation of *Bacillus* sp. DU-106 reduces hypercholesterolemia and ameliorates gut dysbiosis in high-fat diet rats. *Applied Microbiology and Biotechnology*, *105*(1), 287—299. https://doi.org/10.1007/s00253-020-10977-2

Jia, B., Zou, Y., Han, X., Bae, J. W., & Jeon, C. O. (2023). Gut microbiome-mediated mechanisms for reducing cholesterol levels: implications for ameliorating cardiovascular disease. *Trend Microbiol*, *31*(1), 76—91. https://doi.org/10.1016/j.tim.2022.08.003

Majeed, M., Majeed, S., Nagabhushanam, K., Arumugam, S., Beede, K., & Ali, F. (2019). Evaluation of the *in vitro* cholesterol-lowering activity of the probiotic strain *Bacillus coagulans* MTCC 5856. *International Journal of Food Science & Technology*, *54*(1), 212—220. https://doi.org/10.1111/ijfs.13926

O'Morain, V. L., & Ramji, D. P. (2020). The potential of probiotics in the prevention and treatment of atherosclerosis. *Molecular nutrition & food research*, *64*(4), e1900797. https://doi.org/10.1002/mnfr.201900797

Palanivelu, J., Thanigaivel, S., Vickram, S., Dey, N., Mihaylova, D., & Desseva, I. (2022). Probiotics in Functional Foods: Survival Assessment and Approaches for Improved Viability. *Appl. Sci, 12*(1), 455. https://doi.org/10.3390/app12010455

Palaniyandi, S. A., Damodharan, K., Suh, J. W., & Yang, S. H. (2020). Probiotic characterization of cholesterol-lowering *Lactobacillus fermentum* MJM60397. *Probiotics and antimicrobial proteins*, *12*(3), 1161—1172. https://doi.org/10.1007/s12602-019-09585-y

Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Kang, Y., Kim A. R., Holzapfel W., & Ji Y. (2018). Cholesterol-lowering effect of *Lactobacillus rhamnosus* BFE5264 and its influence on the gut microbiome and propionate level in a murine model. *PLoS One*, *13*(8), e0203150. https://doi.org/10.1371/journal.pone.0203150

Plessas, S. (2022). Editorial: Innovations in Functional Food Production: Application of Fruit and Vegetable Juices as Vehicles for Probiotic Delivery. *Front Microbiol*, *13*, 854705. https://doi.org/10.3389/fmicb.2022.854705

Saikia, D., Manhar, A.K., Deka, B., Roy, R., Gupta, K., Namsa, N. D., Chattopadhyay P., Doley R., & Mandal M. (2018). Hypocholesterolemic activity of indigenous probiotic isolate *Saccharomyces cerevisiae* ARDMC1 in a rat model. *Journal of food and drug analysis*, *26(*1), 154—162. https://doi.org/10.1016/j.jfda.2016.12.017

Singhal, N., Maurya, A. K., Mohanty, S., Kumar, M., & Virdi, J. S. (2019). Evaluation of bile salt hydrolases, cholesterol-lowering capabilities, and probiotic potential of *Enterococcus faecium* isolated from rhizosphere. *Frontiers in microbiology*, *10*, 1567. https://doi.org/10.3389/fmicb.2019.01567

Starovoitova, S. O., Kishko, K. M., Bila, V. V., Demchenko, O. M., & Spivak, M.Ya. (2022). Modern aspects of probiotic microorganisms' microencapsulation. *Mikrobiol Z, 84*(5), 72—85. https://doi.org/10.15407/microbiolj84.05.072

Tom, D. H., Schön, C., Wagner, T., Pankoke, H. C., Fluegel, M., & Speckmann, B. (2021). A synbiotic formulation comprising *Bacillus subtilis* DSM 32315 and L-alanyl-L-glutamine improves intestinal butyrate levels and lipid metabolism in healthy humans. *Nutrients*, *14*(1), 143. https://doi.org/10.3390/nu14010143

Yoha, K. S., Nida, S., Dutta, S., Moses, J. A., & Anandharamakrishnan, C. (2022). Targeted Delivery of Probiotics: Perspectives on Research and Commercialization. *Probiotics and Antimicrobial Proteins*, *14*(1), 15—48. https://doi.org/10.1007/s12602-021-09791-7

Yusuf, D., Nuraida, L., Dewanti-Hariyadi, R., & Hunaefi, D. (2019). *In Vitro* Characterization of Lactic Acid Bacteria from Indonesian Kefir Grains as Probiotics with Cholesterol-Lowering Effect. *J Microbiol Biotechnol*, *30*(5), 726—732. https://doi.org/10.4014/jmb.1910.10028

Zhang, F., Qiu, L., Xu, X., Liu, Z., Zhan, H., Tao, X., Shah N. P., & Wei H. (2017). Beneficial effects of probiotic cholesterol-lowering strain of *Enterococcus faecium* WEFA23 from infants on diet-induced metabolic syndrome in rats. *Journal of dairy science*, *100*(3), 1618—1628. https://doi.org/10.3168/jds.2016-11870

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ВІДБІР ПРОБІОТИЧНИХ МІКРООРГАНІЗМІВ ТА ЇХ КОМПОЗИЦІЙ ЯК ОСНОВИ ДЛЯ ЛІНІЙКИ ФУНКЦІОНАЛЬНИХ ПРОДУКТІВ ХАРЧУВАННЯ З ГІПОХОЛЕСТЕРИНЕМІЧНИМИ ВЛАСТИВОСТЯМИ

У сучасній клінічній практиці основними методами корекції підвищеного рівня холестерину в сироватці крові є препарати, які блокують активність ферменту гідроксиметилглутарацил-КоА-редуктази (ГМГ-КоА-редуктази) — статини або препарати, що пригнічують всмоктування холестерину та стеринів у кишечнику (ензатиміб). Усі гіпохолестеринемічні препарати досить дорогі і мають ряд побічних ефектів, основним з яких є гепатотоксичність. Під час моно- та комбінованої терапії інгібіторами ГМГ-КоА-редуктази та ензетимібом можливі випадки підвищення активності аланін- та аспарагінових трансаміназ (біохімічний показник цитолітичного синдрому), розлади травної та дихальної систем, розлади центральної та периферичної нервової систем, розлади органів чуття, збільшення маси тіла тощо. У науковій літературі все більше повідомляється про здатність молочнокислих бактерій знижувати рівень холестерину в сироватці крові. Здатність окремих штамів представників нормальної мікробіоти асимілювати і преципітувати декон'юговані жовчні кислоти, а також руйнувати, зв'язувати і засвоювати холестерин лежить в основі їх гіпохолестеринемічної активності (здатності знижувати рівень холестерину в сироватці крові). Високий рівень холестерину як у загальній сироватці крові, так і в ліпопротеїнах низької щільності є одним із основних факторів ризику ішемічної хвороби серця, атеросклерозу, цереброваскулярного атеросклерозу, гіпертонічної хвороби, пухлин травного тракту тощо. **Метою** дослідження було встановлення гіпохолестеринемічної активності *in vitro* та *in vivo* попередньо відібраних високоефективних пробіотичних штамів молочнокислих бактерій для подальшого створення на їх основі лінійки ефективних функціональних продуктів харчування з гіпохолестеринемічною активністю для профілактики та супутнього лікування патологічних станів, пов'язаних з підвищеним рівнем холестерину. **Методи**. Гіпохолестеринемічну активність бактерій *in vitro* визначали за Rudel L.L., а *in vivo* — на розробленій нами моделі мишей. Досліджено дві схеми введення пробіотичних штамів — профілактичну та лікувальну. **Результати** досліджень показали, що штами *Lactobacillus acidophilus* IMB B-7279 та *Bifidobacterium animalis* IM*B* B-7286, а також композиція *Bifidobacterium animalis* IM*B* B-7286 : *Bifidobacterium animalis* IM*B* B-7285 (1:1) виявилися найбільш ефективними для лікування мишей з гіперхолестеринемією. Слід зазначити, що холестерин-знижувальна активність усіх досліджених пробіотичних штамів та їх композицій коливалася в межах 40—78 %. У той же час слід зазначити, що гіпохолестеринемічна активність інших досліджених штамів була не нижчою, а в деяких випадках навіть вищою, ніж у більшості препаратів, які застосовуються при збільшеному рівні холестерину. **Висновки.** Отримані результати дозволяють стверджувати про необхідність розробки серії функціональних продуктів харчування і пробіотиків на основі досліджуваних штамів та їх композицій в мікрокапсульованому вигляді для профілактики та лікування захворювань, пов'язаних з негативними проявами підвищеного рівня холестерину.

Ключові слова: *функціональні продукти харчування, пробіотики, холестринознижувальна активність, гіпохолестеринемічна активність, холестерин.*