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Probiotics in the creation of fish-based herodietic half-finished products

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ABSTRACT

Feeding is a basic need in human life. The current manuscript presents the first stage in developing of a fish semi-finished product for functional purposes in the production of commercial tilapia fish in high-tech industrial aquaculture. So, the clinical rationale for the probiotic is based on the Escherichia coli 64G strain used in the process of fish breeding for the hero dietic half-finished products manufacturing. So, a has been created to get a functional product (smoked sausage) from fresh tilapia fish grown on specialised feeds at the stage of biotechnological processing. The possibility of using the drug Enterocol as a probiotic strain of Kazakh production is being considered. Probiotics in aquaculture have been used to reduce the level of conditionally pathogenic microbiota in the organs and tissues of fish. This was achieved by Enterocol's action in reducing the organic pollution of water with fish metabolism products at a high stocking density. Probiotics used in industrial aquaculture are an excellent alternative to antibiotics. Moreover, we can get an environmentally friendly product due to probiotics, which is important in creating a healthy food strategy. In an in vivo experiment, authors proved the safety, antagonistic activity, and probiotic proprieties of the *E.coli* 64G strain.

Keywords: functional product, *E.coli* 64G strain, Enterocol, probiotic, herodietic

INTRODUCTION

Modern civilization is focused on a healthy lifestyle. A healthy lifestyle is a healthy diet in which functional products are leading **[1]**, **[2]**. The concept of functional products was developed in Japan. In the conditions of modern civilization, a person eats industrially processed products, and the sedentary lifestyle that has become the norm requires much less energy consumption than physical labor. Under these conditions, food consumption is reduced. Food must be enriched with them to replenish the nutrients necessary for the body **[3]**. The rapid growth in consumption of functional products worldwide indicates that they are the future. Functional products in the modern world are considered an alternative to drug therapy. Functional foods are designed to reduce medication intake. Functional products are not positioned as medicines, but their purpose is disease prevention **[4]**.

The dysfunction of the immune system with age is manifested by an increased susceptibility to infections and a decrease in the ability to respond to vaccinations. In persons over 60 years old, a decrease in the activity of the reaction of secretory intestinal and serum IgA-specific antibodies was found. Changes in the microbiota of the elderly are associated with impaired immune status, characterized by a higher production of pro-inflammatory cytokines (IL-6 and IL-8) in blood plasma. Despite the increased levels of pro-inflammatory cytokines, the reactivity of the innate and adaptive immune systems in the elderly is reduced. In aging, there is a decrease in the activity of the humoral response after vaccination or infection and the formation of T and B cells, natural killer (NK) cells. These changes increase the frequency and severity of infectious, chronic inflammatory,

autoimmune, and oncological diseases **[5]**, **[6]**. The proven efficacy of using probiotics to improve intestines' immune function makes older adults an important target group for probiotic therapy **[7]**.
The most commonly used probiotic carrier is dairy products (cheese, milk, or yogurt) **[8]**. Consumption of the probiotic cheese contributed to the improvement of innate immunity indicators in elderly volunteers: it increased the activity of phagocytosis and the number of NK cells **[9]**. Functional products can improve the gut microflora and, in this way, reduce symptoms of gastrointestinal functional disorders such as irritable bowel syndrome (IBS) **[10]**, which are characterized by impaired intestinal motility and visceral hypersensitivity. Effective treatments for IBS are limited; they provide only partial or short-term relief of symptoms and are often associated with significant side effects. Probiotics can prevent or reduce abdominal symptoms of IBS, such as abdominal pain and flatulence **[11]**.

Abdominal pain in an elderly person is often found in various gastrointestinal tract diseases (including IBS patients) and is associated with visceral hypersensitivity. Probiotics can induce the expression of receptors on epithelial cells that control the transmission of information from the nervous system to the intestine, including opioid (M-, D- and K-) and cannabinoid 2 (CB2) receptors, which can provide analgesic and anti-inflammatory effects **[12]**. World Health Organization shows that 20% of the Kazakh population are elderly people (over 60 years old). According to forecasts 2030, this figure will increase to 25%. In the diet of the population of developed European countries, the proportion of functional products is at least a quarter of their diet. The Kazakh consumer receives, on average, less than 3 kg of functional products per year **[13]**. Developing the local functional products can resolve this case **[14]**.

Scientific Hypothesis

We aimed to study the probiotic strain *E. coli* 64G, which will be a base for the Enterocol supplement drug (probiotic) that will be a component of the functional gerontology dietic semi-finished product. We had to check the hypothesis that the created functional product (semi-finished sausage) could be used in a gerontology diet with the following requirements:

- The functional product has to be a food, not a drug;
- It should be prepared from natural ingredients.
- The content of the component that determines the product belongs to the functional and health-improving group should be at least 1/5 of the body's needs in this food.

So, we wanted to check the ability of the *E. coli* 64G strain to pass the aggressive gastric and intestinal conditions, colonize the intestine, have antagonistic to harmful bacteria action, and be safe for the host organism (laboratory mice). If all these conditions are real, we can make the next trials in creating a safe, efficient, and functional product.

MATERIAL AND METHODOLOGY

The controlled prospective experiment was conducted at the Veterinary Sanitary Expertise Department at the Kazakh National Research Agrarian University, Almaty Technological University, at the Kazakh-Japanese Innovation Center laboratory and the AsylTasEngineering LLP research base.

Samples

The E. coli 64G strain was used to create the Enterocol supplement drug. For testing the proprieties of the probiotic strain, pure microbe cultures (*Salmonella spp*., *Klebsiella spp*., and *Streptococcus spp.*) were isolated from the sick animals in the farms of the Almaty region. *Escherichia coli* 25, *Salmonella typhimurium* 371, and *Klebsiella pneumoniae* 30 were used to test the antagonistic action of the E. coli 64G strain.

Chemicals

In our study, we used nutrient mediums such as meat-peptone agar (MPA) and meat-peptone broth (MPB) produced by the Federal Budgetary Institution of Science "State Scientific Center for Applied Microbiology and Biotechnology" (Obolensk – 142279, Russia). For the *Enterobacteriaceae* family, it has been used Endo agar produced by HiMedia Laboratories, LLC (Mumbai – 400086, India), Ploskirev agar, and HIS-selective medium (Obolensk – 142279, Russia). The Bacillus family has used M-Enterococcus Agar Base (Mumbai – 400086, India) and Blauroccus nutrient medium (Obolensk – 142279, Russia).

For testing proprieties of the *E. coli* 64G strain, hydrochloric acid, bile, and sodium chloride solution (NaCl) were used.

Animals, Plants and Biological Materials

Probiotic Enterocol was experimentally created in the base of the *E. coli* 64G strain *E.coli* was isolated from the healthy lamb intestine and is a normal inhabitant of the gastrointestinal tract. Its identification has been made by the automatic bacterial analyzer in the laboratory of the Kazakh-Japanese Innovation Center in the previous research.

To test the proprietie of the Enterocol we have used laboratory mice. For the study, the preventive efficacy of the Enterokol drug has been used. A total of 55 unbreaded weight mice (14-16 g) were used: 50 of them formed experimental groups, and 5 mice were included in a control one. The white mice from the control group got per os for 1 mL of saline instead of the tested suspension.

Instruments

Autoclav, thermostat, heating cabinet, automatic cell counter Countess® (California, USA), Microbiological analyzer VITEK^{MS} (bioMerieux, USA), and Photocalorimeter KPK-3 (LLP Reaktyvsnab, Shymkent, Kazakhstan); flasks, test tubes, and Petri plates; syringes and pipettes were used in research flowing.

Laboratory Methods

ISO 7218 **[15]** was used as a manual in our research in CFU counting and Bergey's manual of determinative **[16]** was the base for bacteria identification in our experiment. Biological material has been inoculated at the Petri plates on the MPB and Endo mediums and cultivated in a thermostat (t=37°C) for 18 hours.

Morphological: (and tictoral) isolated bacteria features were studied using the Gram method. In case the morphological properties of the enterobacteria were similar, cultures were subcultured onto slanted MPA and Hiss-medium.

Cultural features: were studied in transmitted and reflected light. The crushed drop method was used to check the studied isolates' mobility properties.

Physiological features: were studied at MPB with 0.5% of the corresponding carbohydrate and Andrade indicator. Checking the serogroup E. coli belonging was conducted by using O-coli agglutinating sera. The polyvalent serum and monovalent O- and H-seras were taken to identify the *Salmonella* bacteria group.

Determination of the virulence: of the isolated pure cultures was conducted at the following schema: isolated bacteria were grown on the MPA (t=37-38 °C for 18-20 hours), washed off with the sterile saline, and infested intraperitoneally to the experimental animals in various doses.

The antagonistic activity: of isolated strains was studied on solid nutrient media. The degree of antagonistic activity to each test microbe was checked by the width of the growth inhibition zone of the latter: up to 10 mm - medium, more than 20 mm – high; in the absence of the growth inhibition zone - , the antagonistic activity is missing.

The resistance in bile and hydrochloric acid: was tested in growing *E. coli* 64G on the media with different bile and hydrochloric acid concentrations: meat-peptone agar (MPA) for bile and meat-peptone broth (MPB) for hydrochloric acid with involving photo colorimetric method. Morphological, tinctorial, enzymatic, cultural, pathogenic, and adhesive properties of the isolated bacteria were studied by us.

All bacterial and biochemical studies were conducted under the norms of aseptic and antiseptic.

Description of the Experiment

Sample preparation: The probiotic preparation developed from the selected strain is expected to be used orally. To establish the pathogenicity of *E. coli* 64G strain, 24 h broth and agar cultures were used. Broth cultures *of E. coli* 64G strain in the dosage of 0.5, 0.7, 1.0, 1.2, and 1.5 mL were given orally to white mice.

To determine the harmlessness of the probiotic strain, capturing part of the intestinal chyme and preparing serial 10-fold dilutions in 0.89% NaCl were conducted. One drop of the suspension was taken from each dilution and applied to various nutrient mediums, and the results were carried out in 24 h.

Number of samples analyzed: 21 samples were analyzed in our experiment.

Number of repeated analyses: Biochemical studies were repeated twice. The experimental analyses were repeated three times.

Number of experiment replication: Each experiment has been conducted three times.

Design of the experiment: Morphological, cultural, and biochemical properties of the tested strain were conducted according to common approaches at the different nutrition media: Endo agar, MPA, and MPB (in 24 h and 18 h for the liquid media). The growth temperature range was fixed at 37-39 °C with the optimum at 37 °C. The optimum pH was 6.8-7.5. Glucose, lactose, maltose, arabinose, sorbitol, sucrose, dulcite, and salicin can be used as a carbon source. It forms indole and does not form hydrogen sulfide. It possesses lysine and ornithine decarboxylase activity but does not possess urease activity.

The resistance of the *E. coli* 64G strain to hydrochloric acid and bile was tested according to the biomass accumulation level, changes in the CFU (colony-forming units) number, and pH medium in 18-20 h (for bile medium) and 18 and 24 h for hydrochloric acid medium.

The antagonistic activity: of the *E. coli* 64G strain to the test microbe (*Salmonella spp*., *Klebsiella spp*., and *Streptococcus spp.*) was studied at MNA by the width of the zone of growth retardation of the latter: up to 10 mm - medium, more than 20 mm – high; absence of growth retardation zone - zero antagonistic activity.

The determination of the harmlessness of the probiotic E. coli 64G strain: was studied. In this aim, white mice weighing 16-18 g were infected by the *E. coli* 64G strain. At the pretrial stage, from the mice's

intestinal chyme, 14-18 cultures of Escherichia were isolated with typical cultural and biochemical properties and without hemolytic ability in the bacteriological study. Animals of about the same age were used to obtain comparable results. The results were taken in observing the 50% lethal dose (LD50) for white mice.
The preventive properties of the E. coli 64G strain were studied on mice no later than 30 min after giving oral a geodetic semi-finished product from tilapia fish with the addition of *E. coli* 64G strain (sausages). In the experiment, virulent cultures of *Escherichia coli* 25, *Salmonella typhimurium* 371, *Klebsiella pneumoniae* 30 were used to infect the experimental animals. The experimental animals were infected on the third day by being given the virulent culture orally.

To determine the lethal dose (LD), virulent cultures were titrated on 1.5-year-old mice. 4 experimental animal groups were formed, and the last one was a control. The 20 mice were in each one. To establish the pathogenicity of *E. coli* 64G strain, 24 h broth and agar cultures were used. Each group was introduced to cereal dose of the Enterocol drug: the first – $3x10^9$, 20 mL; the second – $5x10^9$, 20 mL; the third – 10^{10}, 20 mL; the fourth – $2x10^{10}$, 20 mL; and the last (control) group was getting 20 mL of the NaCl (0.89 %). Observation was conducted for 10 days.

Statistical Analysis

Microsoft Excel 2010 and Statistica 15 (USA) were used to test statistically significant differences in our experiment. The presented results are the outcomes of the number of replications experimental results. The differences between groups were taken as significant if $p < 0.05$.

RESULTS AND DISCUSSION

Several hundred thousand functional products are currently recommended to prevent and treat cardiovascular, gastrointestinal tract, and endocrine diseases **[17]**. In looking for future benefits of functional food development, we need to note that it is essential to create functional products for people from certain regions with nutrients most lacking in the diet **[14]**. Elderly people have their need for well-consumed protein and fat components. In this, fish products are one of the best **[18]**. Fish meat (and fish-based products) contains many valuable nutrients like protein, easily digestible fat, vitamins, and minerals **[19]**. The gerontology and heretics development has presented the data that fish from the Cichlidae family, primarily tilapia, contain unique components with neuroprotective properties that are recommended for increased use by elderly people (glycine, chondroitin sulfate, hyaluronic acid, phosphoric calcium salts, phospholipids). That served as the basis for the technology of semi-finished products developed from tilapia fish intended for nutrition in gerontology **[18]**. For example, the recommended daily protein intake of 60-74-year-old men is 85 g, and for women, this rate is 78 g. The daily intake of ascorbic acid is 70-80 mg for both groups. Recently, technologies of canned meat and vegetables, fermented milk drinks, curds, and fish molded semi-finished products have been proposed. However, the list of available specialized products, especially domestically produced ones, is limited **[20]**. It seems relevant and practical to design fish hero-dietary products (and tilapia as a part). So, we had the future task to create a semi-finished fish sausage for introduction in gerontology feeding. In this, we took care that antibiotics used to prevent and treat gastrointestinal diseases are unsafe for the elderly and are becoming less effective.

It is no coincidence that diseases of people over 65, accompanied by diarrheal syndrome, remain the most difficult problem in medicine. Therefore, an important role in preventing intestinal disorders is maintaining an optimal ratio between lactic acid and conditionally pathogenic microorganisms, fungi, and protozoa in the gastrointestinal tract in humans **[21]**. Moreover, probiotic using can reduce the neediness for the antibiotic cure because of the normalized intestine microbiome, so probiotic use makes its income in antibiotic resistance problem **[22]**. Practical experience shows that substitution therapy, aimed at restoring the intestinal microbiome through the regulatory introduction of live bacteria - representatives of normal intestinal microflora, i.e., probiotics, is of great importance in preventing gastrointestinal diseases in people of different ages A probiotic can contain one or more strains of one type of bacteria or several different types **[21]**. The possibility of using multi-species compositions of probiotics assumed that their complex species composition most closely corresponds to the natural composition of normal intestinal microflora. The mechanism of action of probiotics, in contrast to antibiotics, is aimed not at the destruction but at the competitive exclusion of conditionally pathogenic bacteria from the intestinal microbiome to prevent the intensification and transmission of virulence factors in the population of conditionally pathogenic bacteria **[23]**. We were looking for a common spreeded microbe. Kryvda and Rybachuk confirmed our choice of *E. coli* as a common spreeded bacteria **[24]**. The first task was to test the proprieties of *E.coli* 64G strain as a probiotic strain: its bile and hydrochloric acid stability.

Detection of the *E.coli* 64G strain features

At the Endo and meat-peptone agar in 24 h were fixed Escherichia with typical cultural (Figure 1), biochemical, morphological, and tinctorial properties: short rods, motile, gram-negative, do not form spores and capsules.

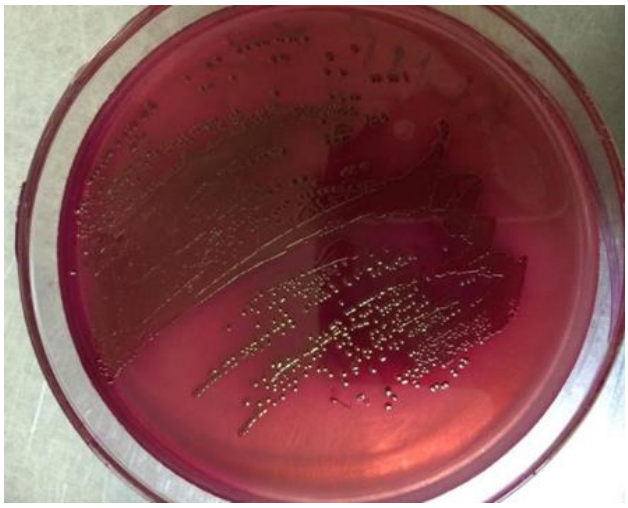

Figure 1 Probiotic *E. coli* strain's growing at the Endo agar: dark red colonies with metal shine.

The cultivation of the *E. coli* 64G strain on the media of MNA (pH 7.0-7.4) for bile and MPB (pH 7.0-7.4) for hydrochloric acid demonstrated the resistance of the tested microbe in them after cultivation for 18-48 h (Figure 2). The control mediums were inoculated by the tested strain, free of the supplement substances.

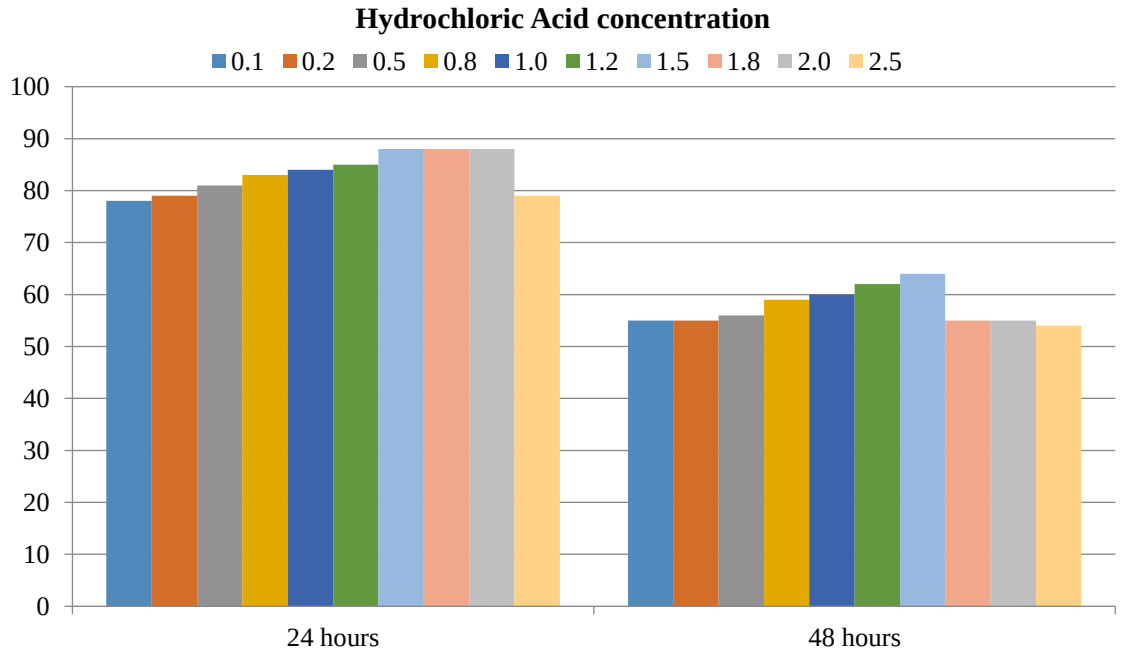

Figure 2 Results of studying the *E.coli* 64G strain's resistance to hydrochloric acid by the photocolorimetric method.

The results of the bile resistance study showed that the *E. coli* 64G strain is highly resistant to 1%, 5%, 10%, and 20% of bile. Escherichia strain has demonstrated high resistance to the various concentrations of hydrochloric acid (0.1%, 0.2%, 0.5%, 0.8%, 1.0%, 1.2%, and 1.5%) (Figure 3).

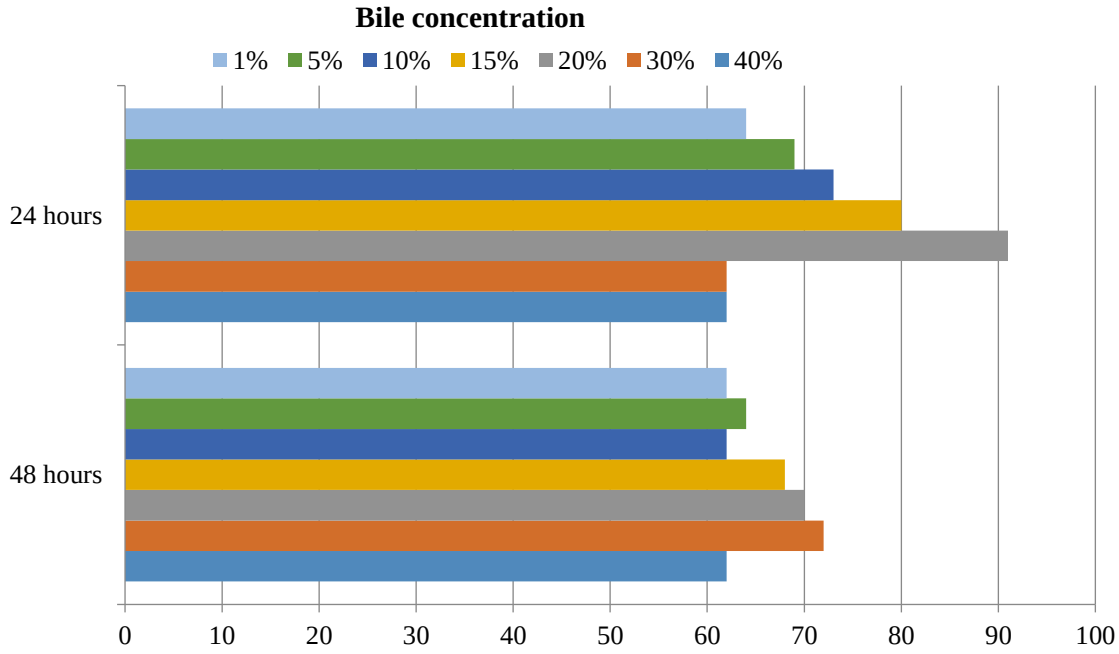

Figure 3 Determination of the sensitivity of the *E. coli* 64G strain to bile

So, tested strain can be passed through the aggressive conditions of the internal tract and can help to reduce age-related changes in immune status, bowel function, and suboptimal nutrition **[25]**. So, it can be used in real conditions of intestinal tract efficiency.

Study the antagonist action of *E. coli* 64G against different bacteria strains

In recent years, many probiotics have been proposed, and many papers characterizing the effectiveness of these drugs **[26]**. And this is important, considering statistical data that 75% of elderly people have different kinds of nutritional disorders **[18]**. In this case, different types of microorganisms and their combinations can be used in their creation, taking into account requirements for probiotic strains that allow them to compete with pathogenic and conditionally pathogenic microorganisms **[26]**. So, the antagonistic activity of the probiotic strain has a significant impact **[27]** and can replace antibiotic therapy in some cases **[28]**. In this way, the other task was - to test the efficiency of the *E. coli* 64G strain in its antagonistic action against pathogen bacteria. In testing the antagonistic activity of the probiotic strain *E. coli* 64G, MNA and the inoculation E. coli 64G strain on the microbe film was used. The results of checking the inhibition zones (mm) can be observed in Table 1. The best inhibition results were observed in *Escherichia coli v3 (6.2 mm)*, *E. coli* v3 (5.8 mm), and *Salmonella abortus ovis* (4.8 mm). The smallest activity was noted against *Klebsiella pneumoniae* v1 (0.2 mm), *Klebsiella pneumoniae* v3 (0.4 mm), and *E. coli* v1 (0.6 mm)

Table 1 Result of the antagonistic activity *E. coli* 64G strain study relation to different bacteria strains.

Tested sample number	Wild strains from sick animals	Diameter of growth inhibition zones, mm
1	*Salmonella dublin*	1.6
2	*Salmonella abortus ovis*	4.8
3	*Salmonella typhymurium*	1.1
4	*Klebsiella pneumoniae* v1	0.2
5	*Klebsiella pneumoniae* v2	1.3
6	*Klebsiella pneumoniae* v3	0.4
7	*Streptococcus pneumoniae*	3.5
8	*Streptococcus pneumoniae* v2	4.3
9	*Streptococcus pneumoniae* v3	3.2
10	*Escerichia coli* v1	0.6
11	*Escerichia coli* v2	5.6
12	*Escerichia coli* v3	6.2

E. coli 64G strain had high antagonistic activity against virulent test cultures. The study found that before and after *E. coli* 64G strain application cultures of *Enterobacteriaceae* family (and *E. coli* as a part), *Enterococci spp.*, *Lactobacillus spp.*, and *Bifidiobacterium spp.* were isolated from the mice's intestines.

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So, the tested strain can be used to normalize the gut microbiome because it is protective against harmful microbe action. There is a lot of data about using *Lactobacillus spp.* **[29]** as well as *Biphidobacillus spp.* **[30]** in normalized gut microbiome. Lee et al. **[29]** gave data that using *Lactobacillus acidophilus* NCFM decreased the visceral pain threshold in an experiment in rats by 44% via the opioid pathway and activated opioid receptors (OR) in humans. Stomach pain is truly actual in elderly people: Satayeva et al. **[18]**, in their last research, gave data that about 20% of elderly are overeaten, 60% of persons eat irrationally (males in the main) with dominant meat meal, flour products, and food with animal fats high content as well as sweets. At the same time, insufficient consumption of dairy products, fish, vegetables, and fruits fixes for these persons. Introducing *Bifidobacterium lactis* Bi-07 can be significantly less effective in increasing OP expression than *Lactobacillus acidophilus* NCFM. Two doses of *L. acidophilus* NCFM at 10^9 and 10^{10} CFU were recommended as clinically appropriate and applicable for use in functional products **[31]**. The effect of L. acidophilus NCFM on IBS symptoms has been determined. In 340 out of 391 volunteers in the groups with initially moderate to severe abdominal pain for 12 weeks of treatment, there was a significant decrease in points on the VAS scale (for probiotics versus placebo, $p = 0.046$). As well as Vinderola et al. **[32]** gave data that oral intake of specific strains of *Lactobacillus* induces the expression of L-opioid and cannabinoid receptors in intestinal epithelial cells and provides an analgesic effect similar to that of morphine. However, using Enterobacteriaceae bacteria is not common. However, there are data about the probiotic activity of *E. coli*. For example, Karim et al. **[33]** studied *E. coli* strains from 40 different samples. The probiotic and antagonist activity was determined for the *E. coli* O157:H7 strain.

But before using each probiotic strain, the safety and efficiency of the drug have been proved in vivo experiments.

Study of the Enterocol safety on the mice organism

Total bacteriological consisting was formed by *Salmonella spp*. (the main part), *Klebsiella spp.*, *E. coli*, and 1-2% of the undifferentiated bacteria of the *Enterobacteriaceae* family. *Enterococci spp.* in the intestinal chyme were mainly represented by *E. faecalis.*

Enterocol introduction has changed the intestinal chyme consisting. In 24 h the Escherichia's number increased by order of magnitude compared to the early study. Escherichia's counts increased 5 and 7 times on the second and third days, respectively ($p < 0.001$). Identifying Escherichia's cultures isolated from the mice's intestines showed that all cultures in their cultural - biochemical, antigenic, and adhesive properties correspond to the *E. coli* 64G strain.

The number of enterococci, lactobacilli, and bifidobacteria in the intestinal chyme of mice 24 h after taking drugs from *E. coli* slightly decreased compared to the control, then on the second and third days after excluding the test *E. coli* 64G strain from the diet increased 1 and 2 times than in the early study. The studies showed that a relatively short-term 2-day use of the bacteriocinogenic strain *E. coli* 64G had a noticeable regulatory effect on the intestinal microflora of mice.

In 48 and 72 h, the enterobacteria's content decreased by 3 and 4 times, respectively. The bacteriocinogenic strains had a significant inhibitory effect on the enterobacteria population. The number of enterococci, lactobacilli, and bifidobacteria in the intestinal chyme of mice on the second and third day increased up to two times.

Also, we have tested the harmlessness of the E. coli 64G strain to the mice's organism after oral income. All animals of the 4 experimental and control groups survived after three oral ingences of the tested probiotic in dosages of 0.5, 0.7, 1.0, 1.2, and 1.5 mL, as well as a control mice's group with the introduction of 1.0 mL NaCl. So, for the 20 days of observation, there were no changes in animal behavior. All white mice were active and mobile, ate well, and had no impaired physiological functions.

So, we can note that the created drug is safe for living organs.

Preventive efficiency of the Enterokol as an antagonistic drug

Prophylactic and therapeutic properties of Enterokol in mice (average age of an adult individual was 1.8 m) were determined by experimental infecting with virulent cultures (*E.coli* 25 strain; *Klebsiella pneumoniae* 30 strain; and *Salmonella typhimurium* 371 strain). In 10 days, mortality was observed for one mouse in the first and the second groups, and 100% of lethality was observed in the control group in all infected mice (Table 2).

Table 2 Preventive properties of the Enterokol tested in mice.

Animal's group	Results of infection with a virulent								
	E.coli 25			*Klebsiellapneumoniae*30			*Salmonella typhimurium* 371		
	Died	Recovered	Survived, %	Died	Recovered	Survived, %	Died	Recovered	Survived, %
First, animals	1	1	90	1	2	85	0	0	100
Second, animals	1	0	90	0	0	100	0	0	100
Third, animals	0	0	100	0	0	100	0	0	100
Fourth, animals	0	0	100	0	0	100	0	0	100
Control, animals	20	0	-	20	0	0	20	0	-

Table 2 data proved the possibility of using *E. coli* 64G strain as a probiotic component, and the best dose was determined to be 10^{10} (20 mL). We can note that that is a good result comparing the data with *Lactobacillus spp.* Frece, in 2005 **[34]**, noted that for *L. acidophilus* M92, *L. plantarum* L4, and *E. faecium* L3, the best-feeding probiotic daily dose was 2×10^{10} CFU. Moreover, we have to note that the antagonistic ability of the E. coli 64G strain, completed by its ability to become the allochthone microbe, makes it applicable to be used as a probiotic strain in functional products. This is actual and essential for elderly people taking care of the fact that in the absence of active microbiota, anticarcia immunity decreases **[35]**. Probiotic components, introduced in the human body by functional products, can significantly improve the patient's immune state **[12]**, **[35]**. These components are capable of activating phagocytes to eliminate early-stage cancer cells **[35]**, and give help in the treatment of necrotizing enterocolitis, colic in infants to constipation, IBS, and hepatic encephalopathy in adult patients **[36]**. Our data allow us to continue our research, taking care of the regulations formulated by Brinton in 1965 **[37]**. But we have to note that this is presented in the beginning research stage, and there is a need for future deep research of the proprieties E. coli 64G strain in manufacturing conditions.

CONCLUSION

The Enterocol drug can supplement the feeding fish diet with later semi-finished products manufactured from fish meat. The results of the studies indicate that the *E. coli* 64G strain, which is the base of the Enterocol, meets certain probiotic requirements: it is non-pathogenic and non-toxic. *E. coli* 64G strain has demonstrated a set of properties that made competing with pathogenic and conditionally pathogenic microorganisms possible. *E. coli* 64G-strain can be transported through the stomach because of its bile and hydrochloric acid resistance. All of these make it possible to use Enterocol like a probiotic. The Enterocol drug can supplement the feeding fish diet with later semi-finished products manufactured from fish meat. The results of the studies indicate that the *E. coli* 64G strain, which is the base of the Enterocol, meets certain probiotic requirements: it is non-pathogenic and non-toxic. E. coli 64G strain has demonstrated properties that made competing with pathogenic and conditionally pathogenic microorganisms possible. *E. coli* 64G strain can be transported through the stomach because of its bile and hydrochloric acid resistance. All of these make it possible to use Enterocol like a probiotic. *E. coli* 64G has demonstrated its activity in the gastrointestinal ecosystem in protecting from semi-pathogen microbes; it can adhere to the epithelium, take root in the digestive tract, and have no pathogen action on the internal organs (confirmed by histological studies). E. coli 64G-strain is stable and can remain viable under production conditions.

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