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The Role of Postbiotic Composition in the Growth Stimulating of Bifidobacteria

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ABSTRACT

Introduction: Probiotic microorganisms are known to increase the biological value of foods, reduce cholesterol levels, positively affect the immune system, prevent intestinal infections and diarrhea associated with antibiotics, reduce symptoms of lactose intolerance, etc. These positive effects depend on the properties of the probiotic strain. The metabolic products of probiotic microorganisms are also capable of having a positive effect on the human body. The metabolic complexes secreted by probiotic bacteria are characterized by high digestibility and resistance to environmental conditions and can potentially be used along with probiotic microorganisms.

Purpose: To study. the possible effect of different concentrations of the postbiotic composition on enhancing the biological properties of the product, in particular, its ability to stimulate the growth of bifidobacteria.

Materials and Methods: As objects of research, a fermented milk product based on a probiotic association consisting of *Lactococcus cremoris* 241C, *Lactocaseibacillus rhamnosus F, Propionibacterium shermanii* E2, developed using a postbiotic complex (PC) in concentrations of 0.5 and 0.01%, was used. In the study of the ability to stimulate the growth of bifidobacteria, a strain of *Bifidobacterium adolescentis* MS–42 from the collection of FGANU «VNIMI» was used as a control culture. The studies were carried out on the GMC 2 medium and a probiotic fermented milk product developed on sterile skimmed milk. The effect of two PC concentrations (0.5 and 0.01%) on stimulating the growth of bifidobacteria in experimental samples after 8 and 24 hours of incubation was studied.

Results: The positive effect of PC at a concentration of 0.01% on the growth of bifidobacteria was confirmed. The absence of a stimulating effect of PC in a concentration of 0.5% may be due to inhibition by acetic acid - the final product

Conclusion: The data obtained allow us to confirm the increase in the biological effectiveness of a fermented milk product with a postbiotic complex in relation to stimulating the growth of bifidobacteria, and recommend it as an additive to the biotechnological system at a concentration of 0.01%.

Keywords: probiotic; postbiotic complex; association of microorganisms; stimulation of growth of bifidobacteria; fermented milk product

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Изучение стимулирования роста бифидобактерий постбиотическим комплексом кисломолочного продукта как один из факторов повышения его биологической эффективности

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АННОТАЦИЯ

Введение: Пробиотические микроорганизмы повышают биологическую ценность пищевых продуктов, снижают уровень холестерина, положительно влияют на иммунную систему, предотвращают кишечные инфекции и диарею, связанную с антибиотиками, уменьшают симптомы непереносимости лактозы и др. Эти положительные эффекты зависят от свойств пробиотического штамма. Продукты метаболизма пробиотических микроорганизмов также способны оказывать положительное воздействие на организм человека. Метаболитные комплексы, секретируемые пробиотическими бактериями характеризуются высокой усвояемостью и устойчивостью к условиям окружающей среды и потенциально могут использоваться наряду с пробиотическими микроорганизмами.

Цель: Изучение возможного влияния различных концентраций постбиотической композиции на усиление биологических свойств продукта, в частности, его способности стимулировать рост бифидобактерий.

Материалы и методы: В качестве объектов исследования использовали кисломолочный продукт, на основе пробиотической ассоциации в составе *Lactococcus cremoris* 241Ц, *Lactocaseibacillus rhamnosus F, Propionibacterium shermanii* Э2, выработанный с применением постбиотического комплекса (ПК) в концентрациях 0,5 и 0,01%. При исследовании способности стимулировать рост бифидобактерий в качестве контрольной культуры использовали штамм *Bifidobacterium adolescentis* МС–42 из коллекции ФГАНУ «ВНИМИ». Исследования проводили на среде ГМК 2 и пробиотическом кисломолочном продукте, выработанном на стерильном обезжиренном молоке. Было изучено влияние двух концентраций ПК (0,5 и 0,01%), стимулировать рост бифидобактерий в экспериментальных образцах через 8 и 24 часа инкубирования.

Результаты: Подтверждено положительное влияние постбиотической композиции (ПК) в концентрации 0,01% на рост бифидобактерий. Отсутствие стимулирующего действия ПК в концентрации 0,5% может быть связано с ингибированием уксусной кислотой - конечным продуктом метаболизма бифидобактерий, которая также входит в состав ПК. Об этом также косвенно свидетельствует и более низкое значение активной кислотности, которая, как известно, является значимым фактором для роста бифидобактерий.

Выводы: Полученные данные позволяют подтвердить увеличение биологической эффективности кисломолочного продукта с постбиотическим комплексом применительно к стимулированию роста бифидобактерий, и рекомендовать его в качестве добавки в биотехнологическую систему в концентрации 0,01%.

Ключевые слова: пробиотик; постбиотический комплекс; ассоциация микроорганизмов; стимулирование роста бифидобактерий; кисломолочный продукт

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INTRODUCTION

As food additives, probiotic bacteria improve gut microbiome and human health by alleviating or preventing such diseases as dysbacteriosis, colitis, dyspepsia, allergies, irritable bowel syndrome, etc. (Havkin, 2021; Islam, 2016; Gareau, 2010; Oelschlaeger, 2010; Martín, 2019). Functional probiotic metabolites improve the secretion and enzymatic processes in the digestive tract, indirectly affecting its biochemical, behavioral, and other physiological functions (Saulnier, 2011; Zobkova, 2023; Agarkova, 2016; Compare, 2017).

The main metabolites of probiotic and lactic acid bacteria include organic acids, e.g., short-chain fatty acids, as well as exopolysaccharides, vitamins, amino acids, enzymes, bacteriocins, etc. Different strains of one and the same microorganism produce a wide diversity of metabolites that have different patterns of synthesis. As part of functional food and nutraceuticals, these metabolites can prevent the risks associated with the consumption of live microorganisms in patients with immune disorders or pancreatic diseases (Mayorgas, 2021; Aguilar-Toalá, 2018; Kaibysheva; 2019).

As dietary supplements, postbiotic complexes have numerous advantages over living cells. In addition to being safe, they have a clear chemical structure and a longer shelf-life; they are easy to dose; they protect their properties from gastric acids and salts in the upper gastrointestinal tract, thus delivering a sufficient count of viable cells (Chistyakov, 2023). Postbiotic complexes start to work immediately after administration, bypassing the three phases that live probiotics have to go through to affect gut microbiota (Szydłowska, 2022; Salminen, 2021). As a popular research topic, postbiotic complexes receive new functional properties and expand the application scope (Teame, 2020). Postbiotic complexes clinically proved their efficiency in preventing and treating various diseases (Cuevas-González, 2020; Nataraj, 2020; Shenderov, 2017). The experimental data on the beneficial properties of postbiotic complexes grow larger in scale and more reliable (Begunova, 2022; Malagón-Rojas, 2020).

Metabolite or postbiotic complexes produced by culturing particular probiotic strains can combine with viable cells of starter associations to produce finished products for people with dysbiotic gastrointestinal health issues. A combination of postbiotic complexes and live probiotic cultures boost the biological effectiveness of such functional products, as well as expand the range of their beneficial effects (Barros, 2020; Oleskin, Shenderov, 2020).

Postbiotic complexes have good prospects as part of foods, beverages, medicines, cosmetics, nutraceuticals, etc. (Donskaya, 2020; Tomar, 2015; Aggarwal, 2022). Global population gets more and more interested in functional foods that not only provide nutrition but also help maintain physical and mental health (Shenderov, 2017). In this respect, postbiotic complexes attract more scientific attention as components of functional foods and nutraceuticals (Chistyakov, 2023). Researchers are busy identifying specific components of postbiotic complexes that are responsible for particular effects on human health. Reuterin produced by *Lactobacillus reuteri* has become one of the first postbiotic compounds with potent antipathogenic activity. It inhibits such intestinal pathogens as salmonella, shigella, proteus, *Pseudomonas aeruginosa*, staphylococci, fungi, and protozoa. Such probiotics keep gut microbiome healthy and maintain the optimal intestinal permeability (Ali, 2022). Bifidobacteria and lactobacilli are the most popular producers of postbiotic compositions. Some studies even explore the possibilities of administering postbiotics to infants because the intestinal barrier and immunity are formed very early in life (Komarova, 2023; Giorgetti, 2015).

A recent study clarified the composition and probiotic potential of the metabolite complex produced by *L. helveticus* H9 (Begunova, 2022). Other researchers proved the antimicrobial, antioxidant, and bifidogenic properties of postbiotic complexes (Rozhkova, 2023). The same team developed a microbial association with probiotic properties that involved *Lc. cremoris* 241C, *L. rhamnosus* F, and *P. shermanii* (Rozhkova, 2021).

In this research, we relied on a study published by Profesor B. A. Shenderov (2017). According to our hypothesis, a postbiotic complex added to a microbial association for probiotic purposes could enhance the biological activity of the final product, in particular, its ability to promote bifidobacteria. We designed a new functional fermented dairy product with various postbiotic concentrations and tested its sensory profile. The concentrations of 0.01 % and 0.5 % provided the best sensory results (Kolokolova, 2024). However, our previous research did not cover the effect of different postbiotic concentrations on the biological efficiency of the functional product, in particular, on its ability to promote the growth of bifidobacteria.

In this study, we researched in-vitro the effect of 0.01 and 0.5 % concentrations of a postbiotic complex on bifidobacteria in the GMK 2 medium of corn and lactose and in a new fermented dairy probiotic product. We highlighted the bifidobacterial promotion with 0.01 and 0.5 % postbiotic complex on the GMK 2 medium and in the fermented dairy product. We also defined the optimal postbiotic concentration to provide the most effective bifidobacterial growth.

STUDY OBJECTS AND METHODS

Objects

The research featured a new fermented dairy product based on a probiotic association of *Lactococcus cremoris* 241C, *Lactocaseibacillus rhamnosus* F, and *Propionibacterium shermanii* E2. The experimental formulation included a postbiotic complex in concentrations of 0.5 and 0.01 %. *Bifidobacterium adolescentis* MS-42 served as control culture. The strain came from the collection of the All-Russian Research Institute of Dairy Industry, Moscow. The GMK 2 nutrient medium of corn and lactose was provided by Biokompas S, Russia.

Methods

The bifidobacterial count procedure was in line with State Standard GOST 33924–2016. All experimental and control samples were subjected to a series of successive tenfold dilutions followed by a triplicate seeding. The resulting cultures were kept in a thermostat at 37 °C. The initial bifidobacterial count took place after 24 h; the final one was performed after 72 h of incubation.

Research Procedure

Preparing the Cultures

L. rhamnosus F and *Lc. cremoris* 241C were activated on sterile skim milk (Komlimilk, Belarus). In case of *P. schermanii* E2, the procedure involved the GMK 2 nutrient medium (Biokompas S, Russia). *Bifidobacterium adolescentis* MS-42 activated on the GMK 2 served as control.

Obtaining the postbiotic Complex

To obtain the postbiotic complex, we added 3 % *L. helveticus* H9 inoculum to the MRS broth (Biokompas S, Russia) to be incubated at (37 ± 1) ºC. To obtain cell-free supernatant, we separated the accumulated cell biomass by centrifugation at 4 °C for 15 min at 6,000 rpm (Rotanta 46, Germany). The resulting supernatant went through a 0.2 µm filter (Sartorius, Germany). The mix was poured into pre-flamed trays, frozen, and dried in a lyophilizer (Labconco, USA). To obtain concentrations of 0.5 and 0.01 %, we collected a certain amount of postbiotic complex under aseptic conditions and diluted it with sterile water.

Preparing the experimental Samples

Microorganisms were added to sterile skim milk (Komlimilk, Belarus) in a ratio of 1:1:6. The postbiotic complex was added simultaneously in the required concentrations. The subsequent cultivation lasted for 8 and 24 h at 37 °C. The control sample contained no postbiotic complex.

Analyzing the stimulating Effect of Postbiotic Complex on Bifidobacteria on the GMK 2 Nutrient Medium

The first stage involved the GMK 2 medium (Biokompas S, Russia) inoculated with 3 % 16-hour culture of B. adolescentis MS-42. The experimental sample contained 0.01 and 0.5 % postbiotic complex. The control sample had no postbiotic complex. The resulting samples were incubated at 37 °C. The bifidobacteria-stimulating effect was assessed after 8 and 24 h of incubation.

Analyzing the Stimulating Effect of Postbiotic Complex on Bifidobacteria in Experimental Samples

At the second stage, we mixed sterile skim milk, 5 % lactic acid microbial association, 3 % of the 16-hour culture of B. adolescentis MS-42, and the postbiotic complex in the same concentrations as at the first stage. A fermented dairy product without postbiotic complex served as control. The resulting samples underwent incubation at 37 °C. The effect of the postbiotic composition on bifidobacteria was assessed after 8 and 24 h of incubation.

Data Analysis

All results were expressed as mean values of three independent experiments. The tables and graphs were constructed using standard Microsoft Office programs. The data were processed using Statistica 10 and MS Excel 2003.

RESULTS AND DISCUSSION

Bifidobacteria-Promoting Ability of Postbiotic Complex on the GMK 2 Nutrient Medium

We tested the postbiotic complex in concentrations of 0.5 and 0.01 % on the GMK 2 nutrient medium after 8 and 24 h of incubation. Figure 1 illustrates the bifidobacteria-promoting effects.

After 8 h of incubation, the sample with 0.01 % postbiotic complex demonstrated a slight increase in bifidobacterial count ($1.0 \cdot 10^8$ CFU/cm^3) compared to the control ($4.0 \cdot 10^7$ CFU/cm^3) whereas 0.5 % postbiotic complex exerted no effect ($6.0 \cdot 10^7$ CFU/cm^3). After 24 h of incubation, the stimulating effect became more obvious. The sample with 0.5 % postbiotic complex had $5.7 \cdot 10^8$ CFU/cm^3; the sample with 0.01 % had $1.0 \cdot 10^9$ CFU/cm^3; the control had $7.0 \cdot 10^7$ CFU/cm^3.

After 8 h, the active acidity was 6.5 pH in the control sample and slightly lower (6.0 pH) in the samples with the postbiotic complex. After 24 h, it was 5.5 pH in the control samples and 4.5 pH in the experimental samples. The experimental samples with different postbiotic concentrations demonstrated no difference.

Bifidobacteria-Promoting Ability of Postbiotic Complex in Experimental Samples

Figure 2 illustrates the changes in bifidobacterial count that occurred in the experimental samples based on the probiotic association and different postbiotic concentrations.

After 8 h of cultivation, the samples based on the probiotic association with and without postbiotic complex had practically the same bifidobacterial count at both concentrations, which varied within $1.0 \cdot 10^8$ CFU/cm^3. After 24 h of cultivation, the sample with 0.01 % postbiotic complex demonstrated an increase in bifidobacterial count, which reached $1.0 \cdot 10^9$ CFU/cm^3. The control sample had $2.0 \cdot 10^8$ CFU/cm^3; the sample with 0.5 % postbiotic complex had $4.9 \cdot 10^8$ CFU/cm^3.

After 8 h, the control and the sample with 0.01 % postbiotic complex showed the same active acidity of 5.5 pH. The sample with 0.5 % postbiotic complex had 5.0 pH units. After 24 h of cultivation, the control had 4.0 pH while the experimental samples demonstrated 4.5 pH. No difference was observed between the samples with different postbiotic complex concentrations.

Figure 1

Bifidobacterial Count in ModelSamples with Different Postbiotic Concentrations

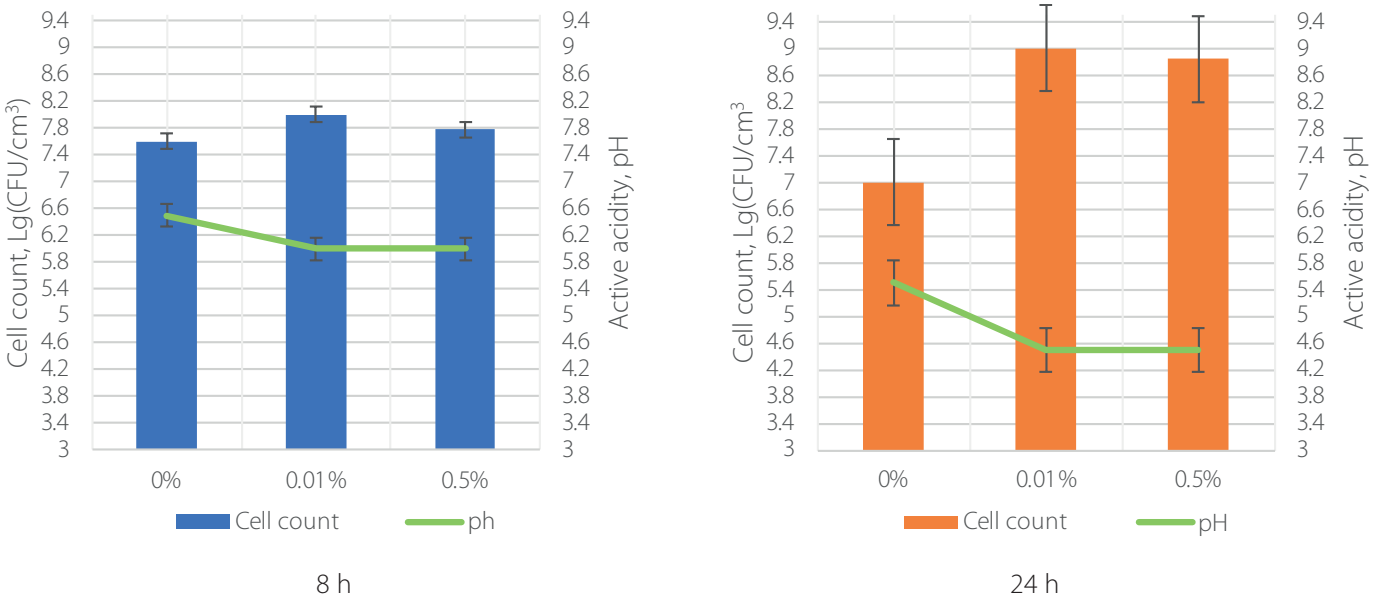
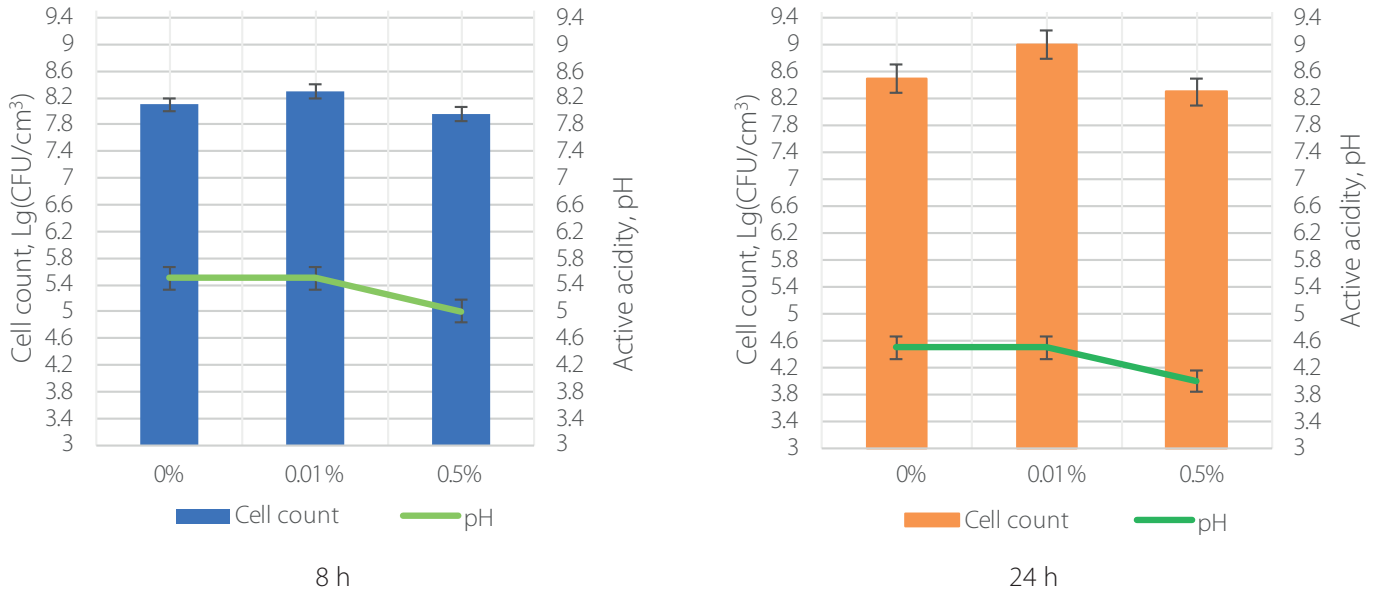


Figure 2

Bifidobacterial Count in Experimental Samples Based on Probiotic Association and Different Postbiotic Concentrations



In this research, the ability to promote the growth of bifidobacteria was the most obvious probiotic property of the postbiotic complex. We found almost no literary data regarding the effect of postbiotic complexes on bifidobacteria when the postbiotic complex was added to the product at the production stage. Lactulose was reported as an oligosaccharide with a proven high prebiotic effect on bifidobacteria (Ryabtseva, 2023). Pectins, which are plant polysaccharides found in apples, were reported as good bifidobacterial promoters. Raw materials containing pectins are a potential source of bifidogenic compounds. Acidic oligosaccharides obtained by pectin hydrolysis increased the bifidobacterial count in the gastrointestinal tract of formula-fed babies. Pectin added to soy milk also stimulated bifidobacteria (Valyshev, 2012). Co-cultivation with other microorganisms, e.g., acetic acid or propionic acid bacteria, promoted bifidobacterial growth due to additional growth factors (Razgulyaeva, 2016). When applied separately, postbiotic complexes and probiotic associations promote the growth of bifidobacteria (Begunova, 2023; Rozhkova, 2021). Based on these data, we attempted to increase the biological efficiency of a new fermented dairy product based on a probiotic association and a postbiotic complex in different concentrations by stimulating the growth of bifidobacteria.

The expected bifidobacterial growth indeed occurred in the samples with 0.01 % postbiotic complex, both on the GMK 2 nutrient medium and in the fermented dairy probiotic product. The bifidobacterial count increased by one order of magnitude, compared to the control. The bifidobacterial growth in the samples with 0.01 % postbiotic complex was more pronounced after 24 h than after 8 h because bifidobacteria have a slow growth rate.

The samples with 0.5 % postbiotic complex caused no bifidobacterial promotion. The much higher concentration had no effect on the development of bifidobacteria because the end products of metabolism, i.e., acetic and lactic acids, inhibited their growth. These acids were part of the postbiotic complex. The low pH in the sample with 0.5 % postbiotic complex was another indirect evidence because pH is known to affect bifidobacteria (Begunova, 2023).

CONCLUSION

The probiotic value of a functional product depends on its ability to stimulate the growth of bifidobacteria. In this research, the biological efficiency of a new fermented dairy product fortified with a postbiotic complex promoted the growth of bifidobacteria. At 0.01 % postbiotic complex, it had the best sensory profile and promoted the bifidobacterial growth, which makes it possible to classify the product as functional. The obtained results can be used to develop an industrial biotechnology for a novel functional probiotic dairy product.

The range of microorganisms that can produce postbiotic complexes can be expanded. Beyond the food industry, postbiotic complexes can be used in cosmetics, nutraceuticals, pharmaceuticals, and agriculture. Further research will reveal the antimicrobial, antioxidant, and angiotensin converting enzyme activities of postbiotic complexes added to fermented dairy products during production.

CONTRIBUTION

Anastasia Y. Kolokolova: wrote the manuscript.

Svetlana A. Kishilova: conducted the experiments and wrote the manuscript.

Irina V. Rozhkova: developed the research concept; set up the objective; wrote the manuscript.

Vera A. Mitrova: conducted the research process; collected the data; visualized the results.

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