



XV International Scientific Conference “Chemistry and Chemical Engineering in XXI century”
dedicated to Professor L.P. Kulyov

Microbiological quality control of probiotic products

A.P. Astashkina^{a,*}, L.I. Khudyakova^a, Y.V. Kolbysheva^a

^a National Research Tomsk Polytechnic University, Tomsk, 634050, Russia

Abstract

Microbiological quality control of probiotic products such as Imunele, Dannon, Pomogayka showed that they contain living cultures of the *Lactobacillus Bifidobacterium* genus in the amount of 107 CFU/ml, which corresponds to the number indicated on the label of products. It is identified that the survival rate of test-strains cultured with pasteurized products does not exceed 10%. The cell concentration of target-microorganisms was reduced by 20-45% after the interaction with living probiotic bacteria. Thus, the yogurt Activia has the most antagonistic activity.

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Peer-review under responsibility of Tomsk Polytechnic University

Keywords: probiotic products, functional foodstuffs, lactobacilli, bifidobacteria.

1. Introduction

Environmental deterioration, stress, eating disorders (deficiencies of plant food, vitamins, synthetic substitutes) led to the development and practical implementation of the concept "probiotics and food products", or foodstuffs, developed in the last decade of the 20th century¹. The term "probiotic" refers to a product based on intestinal commensals which are capable of implementing a biological control in the body and have regulatory and trigger properties¹.

Probiotics contain live lactic-acid bacteria belonging to the most typical representatives of the normal human intestinal microflora (lactobacilli, bifidobacteria, colibacteria)¹⁻¹¹. The list of probiotic microorganisms that may have positive health effects is quite extensive. All variety of probiotics, depending on the functional activity, are

* Corresponding author, tel: +79234128286
E-mail: apa2004@mail.ru

divided into four generations¹⁻². An analysis of the literature¹⁻⁴⁷ showed that probiotics are used for correcting microecological disturbances of acute and chronic diseases such as dysfunction of the gastrointestinal tract^{4,6,26-29}, diseases of the cardiovascular system^{38,43}, and metabolic disorders after antibiotic, hormone and radiation therapy. Probiotics are also used in surgical practice in the preoperative and postoperative period, in gynecology for correcting dysbiotic states of the genital tract of women^{1,22,28}, and in cases of dysimmunity^{8,16}, allergic diseases and others^{1,31,38}. Biological potential of probiotics is primarily due to an antagonistic activity, associated with the synthesis of lactic and acetic acids, as well as other numerous metabolic effects, including the synthesis of vitamins B, K and of short-chain fatty acids (SCFA), the dietary carcinogen inactivation, the bacterial fermentation of certain drugs, the synthesis of signal molecules, etc.^{8,31,43,44}. Besides the above areas of a prophylactic application of probiotics, currently, some prerequisites were outlined for the expansion of their use, and, in particular, for the reduction of blood serum cholesterol⁴⁵, prevention of saproductia⁴⁶ and nosocomial diarrhea^{47,48,49} etc.

Today in Russia there are about 100 registered probiotics, regulating a balance of intestinal microflora, that are based on living organisms or their metabolic products^{7,30,38,43,44}. Every year, new food additives and food products containing probiotic microorganisms are produced on the market^{1,9,16,44}. However, the claim on the label stating that the product contains living micro-organisms in a large amount is not always true. Furthermore, the very presence of probiotic microorganisms in the products is not evident of their functional efficiency; thus, the ability of such products to inhibit "harmful" bacteria cannot simply be declared^{1,2}.

In this regard, the purpose of the work was to conduct microbiological quality control of probiotic foods – namely, to establish belongings of microorganisms to *the Lactobacillus and Bifidobacterium genus*, and to determine the quantitative content of living probiotic microorganisms and the antagonistic activity of probiotic cultures.

2. Materials and Methods

For this research, the following functional foodstuffs containing probiotic cultures were examined: the drinkable yogurt "Activia" (company Dannon, manufactured in Russia, settlement Lyuuchany) containing *Bifidobacterium actiregularis*; the drinkable yogurt "Dannon" (company Dannon, manufactured in Russia, settlement Lyuuchany) containing *Lactobacillus casei*; the drinkable yogurt "Imunele" (company Wimm -Bill- Dan, manufactured in Russia, city Moscow) containing *Lactobacillus casei* and *Lactobacillus rhamnosus*; and the children's porridge "Pomogayka" (company Nestle, manufactured in Russia, city Moscow) containing *Bifidobacterium sp.* These probiotic products were stored at a temperature not exceeding 4⁰C, following all conditions of proper storage.

To study the antagonistic activity as targets, the following microorganism strains were examined: *Staphylococcus aureus* 668, *Proteus vulgaris* 588, *Salmonella typhimurium* 59-60 (representative office "Medgamal" of the Scientific Research Institute of Epidemiology and Microbiology (SRIEM) named after N. F. Gamaleya, Russian Academy of Medical Sciences (RAMS), Moscow).

To isolate, cultivate and examine the properties of lactobacilli and bifidobacteria, the media produced by the firm Himedia (India) were used. They are presented in Table 1.

Table 1. Culture media.

Name of medium	Abbreviation	Purpose
<i>Lactobacillus MRS Broth</i>	MRS	Isolation of lactobacilli
<i>Lactobacillus MRS Agar</i>	LBA	Identification and quantitative account of lactobacilli
<i>Bifidobacterium Broth</i>	BBB	Isolation of bifidobacteria
<i>Bifidobacterium Agar</i>	BBA	Identification and quantitative account of bifidobacteria
<i>Nutrient Broth</i>	NB	Determination of antibiotic activity
<i>Nutrient Agar</i>	NA	Cultivation of strains of test microorganisms

To isolate the bacteria lactobacilli and bifidobacteria from probiotic food products, the product culture method of

selective media was used. Inoculations were incubated for 48 hours at a temperature of 37°C in microaerophilic conditions in packages «Gaspak» produced by the company "Himedia" (India).

The categorization of selected isolates into *the Lactobacillus and Bifidobacterium genus* was conducted on a cultural-morphological basis (growth characteristics and cell morphology) and physiological and biochemical characteristics (formation of catalase enzyme). Cell morphology (shape, location, mobility, and presence or absence of spores belonging to the Gram-stain) was studied using a laboratory microscope "Biolam" M (produced by the open joint-stock company (OJSC) "LOMO", Russia) according to generally accepted practice in microbiological techniques.

The determination of total lactic-acid bacteria in functional foodstuffs was performed by the method of Koch. The inoculation on solid media was done in dilutions 1:10⁵, 1:10⁶ in duplicate.

To study the antagonistic action of probiotic products on opportunistic-pathogenic bacteria, the method of product co-culture with test-strains was used. The proportions of ingredients were as follows: 0.1 g of a product suspension and 1.0 ml of a test-culture containing 10⁸ cells in 1 ml. The cultivation was carried out for 2 hours in a thermostatic shaker at a temperature of 37°C with a rotation speed of 220 rpm (thermostat-shaker WiseCube, Germany). The inhibitory effect of the product was evaluated by the survival rate of a test-microorganism at a co-cultivation in the medium, containing a probiotic product.

3. Results and Discussion

The isolation and identification of bacteria in probiotic products was carried out on selective media with a subsequent determination of cultural and morphological properties. In the study of morphological characteristics in products with lactobacilli (Imunele, Dannon), small cream-colored colonies with a smooth edge and convex shape were found. Being gram-positive and catalyst-negative, they have a fine-grained structure with the characteristic odor of sour milk. In a solid medium with probiotic products Activia, Pomogayka, containing bifidobacteria, gram-positive asporogenous (nonspore-forming) colis with a flat or slightly curved shape were found. The ends of the colis of the majority of bifidobacteria are forked but can also be refined or thickened in the form of spherical bubbles. The length varies from 0.5-1.3 – 1.5-8.0 µm. In the semi-solid medium, isolated colonies in the form of studs or sticks are formed. On the basis of culture-morphological (growth characteristics and cell morphology) and physiological and biochemical characteristics it is concluded that the isolated cultures belong to *the Lactobacillus and Bifidobacterium genus*.

According to the modern requirements, probiotic bacteria in these products must be alive and in a large number (at least 10⁶-10⁷ CFU in 1 g of a product). These requirements relate to the fact that most of these bacteria are killed in the stomach and duodenum⁵. Although probiotics, included in the ferment structure, are specifically tested for resistance to gastric acid and bile, only 5-10% of living bacteria reaches the large intestine³. However, such products do not always contain the number of bacterial cells which is indicated on the label⁴. In this regard, the quantitative content of beneficial bacteria was examined in probiotic products. The inoculation was done within two days of purchasing the product. Results determining the number of living cells in the tested products are presented in Table 2.

Table 2. Contents of lactobacilli and bifidobacteria (CFU/g) in functional foodstuffs.

Product	The number of living probiotic bacteria in 1g of the product	
	Stated number	Actual number
Pmogayka	10 ⁶	2x10 ⁷
Imunele	10 ⁷	5x10 ⁷
Activia	10 ⁷	3x10 ⁷
Dannon	10 ⁷	3x10 ⁷

It was determined that the content of probiotic bacteria in the studied products fully meet their quantity indicated on the label of products. However, the presence of living probiotic bacteria in food does not guarantee their efficiency, the majority of which is intermicrobe antagonism.

Antagonistic properties of lactobacilli are the basis for the creation of probiotics, which are a physiological means of suppressing excessive reproduction of opportunistic-pathogenic microorganisms, as well as the correction of the number of individual components of the resident microflora at dysbacterioses. The formation of lactic and acetic acid, lysozyme, hydrogen peroxide, and bacteriocins inhibit the generation of other bacteria, including enteric pathogens and opportunistic-pathogenic bacteria in the process of metabolism of lactobacilli and bifidobacteria. As target-microorganisms, *Staphylococcus aureus*, *Salmonella typhimurium* (food poisoning germs), and *Proteus vulgaris* (aerobic rotting pathogen) were selected. The presence of these bacteria in food indicates that they are unsafe to eat; the realization of such products is prohibited.

To determine antagonistic abilities, the method of co-cultivation of the above three test-cultures in culture fluid MRS-5 with selected products was used. The inhibitory effect of probiotic products is determined by the level of survival rate (%) of test-strain cells (Table 3).

Table 3. The antagonistic activity of probiotic products and their probiotic components co-cultured with the test-organisms.

Product	Variant of experiment	Survival rate of test-strain cells, %		
		<i>Staphylococcus aureus</i> 668	<i>Proteus vulgaris</i> 588	<i>Salmonella typhimurium</i> 59-60
Activia	Raw product	63	70	58
	Pasteurized product	90	87	88
Imunele	Raw product	82	85	80
	Pasteurized product	96	92	96

As can be seen from the data, after pasteurization, the product has almost no inhibitory effect on the growth of the test-strains. The survival rate of test-strains when cultured with pasteurized products is no more than 10% different from the control. A negative effect was only evident in the original product which contained living cultures. The cell concentration of target-microorganisms after the interaction with living probiotic bacteria was reduced by 20-45%. The yogurt Activia has the most antagonistic activity, which is composed of living bifidobacteria. In this case, test-microbe survival is 63% for *S.aureus*, 70% for *P.vulgaris* and 58% for *S.typhimurium*.

4. Conclusions

During the microbiological control of functional foodstuffs, it was determined that drinkable yogurts such as Activia, Imunele, and Dannon contain living lactobacilli and bifidobacteria in the amount of 10^7 CFU/ml. In the children's porridge Pomogayka, bifidobacteria are found at a titre of 10^6 CFU/g. The content of probiotic bacteria in these products fully meet their quantity indicated on the product label. The identification by culture-morphological, physiological and biochemical characteristics supports that selected isolates belong to the *Lactobacillus Bifidobacterium* genus. It is identified that the survival rate of test-strains cultured with pasteurized products is not more than 10% different from the control ones. Most antagonistic activity has a probiotic product Activia.

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