

The use of probiotic cultures in the technology of protein fermented milk products

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Abstract. The article provides a brief justification for the need to create new fermented milk products with probiotic properties based on microbial consortia consisting of thermophilic lactic acid streptococcus, propionic acid bacteria and bifidobacteria. The biochemical activity of consortia has been studied. Data on the synthesis of vitamin B12 in various microbial consortia are presented. A high level of viable cells of propionic acid microorganisms and bifidobacteria was shown when co-cultured with thermophilic streptococcus. The results of the conducted studies have shown the possibility of using microbial consortia consisting of thermophilic lactic acid streptococcus, propionic acid bacteria and bifidobacteria for the production of protein fermented dairy bioproducts.

1 Introduction

The microbiome located in the human gastrointestinal tract (GI tract) includes the largest community (diverse and dense) of bacteria and, in combination with a favorable internal environment, contributes to the production of regulated anti-inflammatory signals in the gastrointestinal tract, which contributes to immunological and metabolic tolerance and a significant effect on human physiology [1, 2]. Microbiome research, which focuses on the behavior, interactions, and functions of microbial communities in a particular environment, has achieved tremendous success over the past 15 years [3]. Functional diversity, not taxonomic, is a much more fundamental and significant characteristic that emphasizes the state of the microbiome [4].

Among the known probiotic microorganisms beneficial to health, bifidobacteria are one of the most dominant groups, and some types of bifidobacteria are often used as a probiotic ingredient in many functional foods [2].

The numerous medical benefits associated with bifidobacteria and the subsequent commercial significance due to their inclusion in functional foods have led to increased research aimed at a molecular understanding of the claimed properties of probiotics of this genus [5].

The end products of metabolism, such as vitamins, polyunsaturated fatty acids, conjugated linoleic acid, contribute to the functionality of the intestine and the probiotic properties of bifidobacteria. The end products of bifidobacterial fermentation of complex carbohydrates

in the gastrointestinal tract play a key role in human metabolism, as they contribute to the integrity of the epithelial barrier, produce antibacterial compounds, ferment indigestible fiber, stimulate the absorption of water and sodium, reduce the pH of the lumen and bioavailability of toxic amines, promote the production of amino acids and vitamins, prevent colonization of pathogenic microorganisms [6, 7, 8, 9]. There is evidence that acetate produced by bifidobacteria can enhance intestinal protection by epithelial cells and thereby protect the host from *E. coli* O157:H7 infection [10].

In 2015, at the Congress of the Federation of European Microbiological Societies (FEMS), the concept of including multicomponent probiotic consortia in food was put forward. With regular use, probiotics regulate and stimulate digestion, enhance immunity, increase colonization resistance of the intestine, and prevent the development of allergic complications. The effectiveness of multicomponent probiotic consortia depends on many factors, including human physiological characteristics, the presence of chronic and acute diseases, etc. [11].

According to experts, one of the innovative directions of food biotechnology development is the development of biotechnological approaches to the production of probiotics, synbiotics, starter cultures, new strains of lactic acid and other technological microorganisms, microbial consortia with specified biological properties and optimized technological characteristics [12]. Dairy bioproducts enriched with

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lactic acid microflora and probiotic cultures are created on the basis of starter consortia.

All over the world, work is underway on the selection and research of new probiotic strains of bifidobacteria and propionic acid microorganisms characterized by high antagonistic and biochemical activity, increased resistance to adverse factors.

At the same time, much attention is paid to the construction of microbial consortia based on new or already known strains. A consortium is an association of two or more microorganisms, as a rule, with similar biochemical and technological properties in the absence of antagonism between cultures [13].

The use of optimally selected consortia allows us to ensure the desired probiotic, organoleptic, physico-chemical and technological properties of the finished product. As a rule, microbial consortia are able to grow on more complex substrates and have increased resistance to the effects of bacteriophages, growth inhibitors and other adverse factors [14].

1.1 The purpose of the work

The purpose of this work is to create protein fermented milk products based on starter cultures, which include probiotic strains. To do this, we studied the technological parameters of the production of fermentation and processing of the clot and the qualitative characteristics of the resulting bio-curd.

2 Methodology and materials

Experimental studies were conducted at the Department of Dairy Products Technology. Commodity Science and Examination of Goods", the problematic research laboratory of the East Siberian State University of Technology and Management.

A strain of propionic acid bacteria *Propionibacterium freudenreichii subsp.* was used in the work. *Shermanii AC-2503*, a freeze-dried starter culture of direct application DANISCO CHOOZITM TA 61 LYO 50 DCU, which includes thermophilic lactic acid *Streptococcus thermophilus*.

The strain of propionic acid bacteria was obtained from the fund of the All-Russian Collection of Microorganisms of the Institute of Biochemistry and Physiology of Microorganisms (Moscow), activated by a biotechnological method developed at the East Siberian State Technological University. For the research work, a bacterial concentrate of bifidobacteria was used, which includes the strain *Bifidobacterium longum B379M*. The manufacturer of bifidobacteria concentrate is a small innovative enterprise "Bifivit" in Ulan-Ude.

The main physico-chemical and microbiological parameters of raw materials, starter cultures and fermented milk products were determined by standard and generally accepted methods in research practice

Table 1. Characteristics of consortia.

Indicator	Characteristic		
	Sample 1	Sample 2	Control
Composition	<i>Streptococcus thermophilus</i> , <i>Propionibacterium freudenreichii subsp. Shermanii AC-2503</i>	<i>Streptococcus thermophilus</i> , <i>Bifidobacterium longum B379M</i>	<i>Streptococcus thermophilus</i>
The ratio of cultures	1:1	1:1	-
Duration of fermentation, hour	8	10	9,0
Titred acidity, °T	78	72	76
pH	4,75	4,83	4,75
The amount of vitamin B ₁₂ , mcg/ml	35,40	0,47	0,45
The number of cells of <i>Propionibacterium freudenreichii subsp. Shermanii AC-2503</i> , κ.o.e./cm ³	10 ⁹	-	-
Number of <i>Bifidobacterium longum</i> cells in 379M, κ.o.e./cm ³	-	10 ⁹	-
The number of cells of lactic acid bacteria, κ.o.e./cm ³	10 ⁹	10 ⁹	10 ⁹

3 Results and Discussion

The essence of most technological techniques is reduced to regulating microbiological processes by setting certain temperature conditions, since the quality of the finished product depends on them.

In order to choose the right temperature regimes, it is necessary to study in detail the physico-chemical, biochemical processes, taking into account organoleptic parameters. An important factor affecting the growth of microorganisms is the indicators of titrated and active acidity. The reaction of the medium can change the activity of enzymes, which in turn leads to a change in the biochemical activity of microorganisms that cause certain transformations in the medium [17].

Three samples of starter culture consortia were prepared. A starter culture based on *Streptococcus thermophilus* was used as a control. The composition of the consortia and their characteristics are presented in table 1.

Biochemical activity, the amount of synthesized vitamin B₁₂ and microbiological parameters were taken into account in all samples. It can be seen from the table data that good organoleptic characteristics, homogeneous, dense consistency, mild refreshing fermented milk taste, fermentation duration is (8-10) hours were noted in all samples. A sample of sourdough with propionic acid bacteria is characterized by higher active acidity and a decrease in fermentation time.

Cultivation temperature is one of the main physical factors regulating biological processes. Subsequently, the cultivation of starter culture consortia was carried out at various temperatures of 35 °C, 37 °C, 39 °C and the effect of temperature on the dynamics of acid formation and quantitative growth of probiotic cultures was studied. The results of the studies are presented in Table 2.

The analysis of the obtained data indicates that the cultivation temperature of 37 °C stimulates the active development of probiotic microorganisms. It should be noted that at a temperature of 37 °C, the duration of fermentation decreases in all samples. An increase in the fermentation time is observed in the sample with bifidobacteria. An increase in the fermentation temperature to 39 °C helps to reduce the duration of clot formation and is (9.5-10.5) hours, but there is an inhibition of the growth of probiotic cultures and lactic acid microorganisms. Taking into account the revealed dynamics of clot formation and the growth of probiotic microflora, the most optimal temperature regime is 37 °C.

The experimental data obtained indicate that the new protein probiotic products are characterized by good organoleptic characteristics – they have a pleasant fermented milk taste, a soft, crumbly consistency and contain a high number of cells of bifidobacteria and propionic acid bacteria 10⁷ k.o.e./cm³.

The process of storage capacity of fermented dairy bioproducts has been studied. During storage, a slight increase in acidity was noted in the studied samples. It is very important that when storing all production samples, a high number of viable cells of probiotic cultures and a low level of development of sanitary-indicative microflora are preserved. This is due to the fact that during storage, probiotic and lactic acid microorganisms synthesize a significant amount of antibiotic substances that prevent the development of E. coli bacteria, yeast

and mold. The optimal shelf life of the bio-curd is 7 days at a temperature of 4-6 °C.

Table 2. Dynamics of acid formation and quantitative accounting of probiotic cultures depending on the cultivation temperature

The indicator at a fermentation temperature of 35 °C	Characteristics of the samples		
	Sample 1	Sample 2	Sample 3
Duration of fermentation, hour	11	12,5	12
pH	4,56±0,03	4,69±0,03	4,60±0,03
The number of cells of <i>Propionibacterium freudenreichii subsp. Shermanii AC-2503</i> , k.o.e./cm ³	4×10 ⁷	-	-
The number of cells of <i>Bifidobacterium longm B379M</i> , k.o.e./cm ³	-	5×10 ⁷	-
The number of cells of lactic acid bacteria, k.o.e./cm ³	10 ⁷	10 ⁷	10 ⁷
The indicator at a fermentation temperature of 37 °C			
Duration of fermentation, hour	9,5	10,5	10
pH	4,54±0,03	4,58±0,03	4,56±0,03
The number of cells of <i>Propionibacterium freudenreichii subsp. Shermanii AC-2503</i> , k.o.e./cm ³	6×10 ⁷	-	-
The number of cells of <i>Bifidobacterium longm B379M</i> , k.o.e./cm ³	-	9×10 ⁷	-
The number of cells of lactic acid bacteria, k.o.e./cm ³	10 ⁷	10 ⁷	10 ⁷

Table 2. Part two

The indicator at a fermentation temperature of 39 ° C			
Duration of fermentation, hour	8,5	10	9,5
pH	4,36±0,03	4,46±0,03	4,43±0,03
The number of cells of <i>Propionibacterium freudenreichii subsp. Shermanii AC-2503</i> , k.o.e./cm ³	8×10 ⁶	-	-
The number of cells of <i>Bifidobacterium longm B379M</i> , k.o.e./cm ³	-	2×10 ⁶	-
The number of cells of lactic acid bacteria, k.o.e./cm ³	10 ⁶	10 ⁶	10 ⁶

The process of storage capacity of fermented dairy bioproducts has been studied. During storage, a slight increase in acidity was noted in the studied samples. It is very important that when storing all production samples, a high number of viable cells of probiotic cultures and a low level of development of sanitary-indicative microflora are preserved. This is due to the fact that during storage, probiotic and lactic acid microorganisms synthesize a significant amount of antibiotic substances that prevent the development of *E. coli* bacteria, yeast and mold. The optimal shelf life of the bio-curd is 7 days at a temperature of 4-6 ° C.

4 Conclusion

Symbiotic starter cultures based on thermophilic lactic acid streptococcus, propionic acid bacteria and bifidobacteria have been developed. It has been established that there are no antagonistic manifestations in the joint cultivation of propionic acid bacteria and bifidobacteria with thermophilic lactic acid streptococcus.

The high biochemical activity of combined starter cultures was noted. The developed associations of microorganisms make it possible to obtain protein fermented milk products with a high content of viable cells of probiotic cultures. The shelf life of protein fermented dairy bioproducts has been studied. Their guaranteed storage period is 7 days at a temperature of 4-6 ° C.

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