Вивчено жирнокислотний склад бактеріальних заквасок у монокультурі та у консорціумі біфідобактерій та пропіоновокислих бактерій. З досліджуваних зразків відрізнялася своїм жирнокислотним складом закваска симбіотичного консорціума B.longum-Я3 і P.shermanii-PS. В цьому зразку сума ненасищених жирних кислот склала 78,36 %, а кількість лінолевої кислоти – 23,99 % від загальної суми жирних кислот

Ключові слова: пропіоновокислі бактерії, біфідобактерії, насичені жирні кислоти, ненасичені жирні кислоти

Изучен жирнокислотный состав бактериальных заквасок в монокультуре и в консорциуме бифидобактерий и пропионовокислых бактерий. Из исследуемых образцов отличалась своим жирнокислотным составом закваска из симбиотического консорциума B.longum-Я3 и P.shermanii-PS4. В этом образце сумма ненасыщенных жирных кислот составила 78,36 %, а количество линолевой кислоты – 23,99 % от общей суммы жирных кислот

Ключевые слова: пропионовокислые бактерии, бифидобактерии, насыщенные жирные кислоты, ненасыщенные жирные кислоты

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RESEARCH INTO FATTY ACID COMPOSITION OF PROBIOTIC CONSORTIUMS WITH THE INCLUSION OF PROPIONIC ACID BACTERIA

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1. Introduction

In recent years, many scientists have paid considerable attention to the concept of recovery and rejuvenation of the human body with the help of including in the ration of sour-milk products that contain probiotic cultures [1].

The action mechanism of probiotics is predetermined by both the high content of viable cells and the accumulation of their extracellular metabolites, which strengthen the probiotic effect. Most attention is paid to the microbial synthesis of polyunsaturated fatty acids (PUFA). They possess the anticarcinogenic, antigenogenic and antioxidant properties, they are the immunnomodulators, and control increase in the deposit fat [2]. PUFA also regulate a multitude of physiological processes in the human organism [3, 4].

Scientists have always paid considerable attention to the role of fatty acids in human nutrition. Fatty acids were introduced into the ration of user by means of BAD rich in PUFA of plant and animal origin. However, until now, when constructing the technology of BAD, the possibility to use PUFA of the microbial synthesis has not been addressed. That is why examining the fatty-acid composition of probiotic cultures is a relevant task of biotechnology.

2. Literature review and problem statement

At present, more and more scientists have been paying attention to studying the fatty acids and their role in human nutrition [3, 5, 6]. It is known that the delivery of the saturated fatty acids and polyunsaturated fatty acids is equally necessary for the human organism; they also participate in the same biochemical reactions. It is important to note that there is a feedback between the concentration of cholesterol in the human blood and the content in the food ration of PUFA. The ω-3 PUFA reduce general cholesterol content in the blood, first of all due to its most atherogenic fraction – the lipoproteins of low density (LPLD) and increase the content of lipoproteins of high density (LPHD). In addition, atherogenic properties are demonstrated by the ω-6 PUFA, which strengthen the regeneration of tissue while their derivatives are the most important regulators of immune status. Thus, averting the atherosclerotic damage of vessels is possible through the systematic application of products of probiotic designation [4].

In all living organisms, the biosynthesis of PUFA proceeds into two stages. Two ferments are necessary for this process – elongase and desaturase [7]. Desaturase transforms the C-C bond into C=C. Elongase lengthens the carbon chain by 2 carbon atoms, linking them to the carboxyl molecule end. At the first stage there occurs the *de novo* synthesis from acetate of short-chain and medium-chain fatty acids, which concludes with the formation of palmitic acid. Next, with the participation of elongase and desaturase, there occurs a sequential formation of stearic (C18:0) and oleic acids (C18:1ω-9). Then comes the second stage of the biosynthesis, which varies in the different groups of organisms [8].

Microorganisms and some invertebrates have many ferments, which possess elongase and desaturase activity, necessary for the *de novo* synthesis of various PUFA, and which are the primary producers of these substances [2, 8, 9]. Oleic acid in them with the help of desaturase Δ12 is transformed into linoleic acid, which is the main predecessor of the biosynthesis of PUFA. Next, the microorganisms convert linoleic acid into the more long-chain fatty acids by a series of the reactions of desaturation and elongation [10].

Humans and animals are not capable of synthesizing linoleic acid from oleic acid. In the human organism there are only Δ4, Δ5, Δ6 and Δ9 desaturases, while the ferment Δ12 desaturase is absent. This is why more long-chain PUFA are not capable of being synthesized *de novo* in the human organism. Thus, a balanced amount of the given fatty acids must enter human organism from different plant or microbial food sources [8].

Bacteria of the genus *Propionibacterium* are the representatives of obligatory micro-biota of the rumen of ruminant animals. These bacteria convert linoleic acid into different isoforms of the conjugated linoleic acid (CLA). The formation of CLA isoforms is accomplished by the means of biohydrogenation. Due ti this process, milk and meat of ruminant animals are the basic food sources of the unsaturated fatty acids.

Such microorganisms as Bifidobacterium, Lactobacillus, Enterococcus also possess the isomerase of linoleic acid, which allows them to form the unsaturated fatty acids as the mechanism of detoxication [11, 12]. That is why the development of BAD and starter cultures with the use of such microorganisms is an important biotechnological task. This product will be able to manifest both a probiotic effect and will be capable of improving the level of essential fatty acids and the biological value of fermented products.

3. The aim and tasks of research

The aim of present research is to study changes in the fatty acid composition of bacterial starter cultures in the monoculture and in the consortium of bifido- and propionic acid bacteria. To prove the possibility of enrichment of finished products with essential fatty acids using the above-indicated microorganisms.

To accomplish the set aim, the following tasks were formulated:

– to explore the accumulation of the biomass of the bacteria considered in present work both in the monoculture and in the consortium on a special nutrient soybean- lactose medium;

– to determine the fractional composition of fatty acids of the examined samples.

4. Materials and methods for examining the fatty acid composition of probiotic consortia with the inclusion of propionic acid bacteria

4. 1. The examined materials, utilized when conducting the study, and technique for conducting the experiment

In the present work we used the cultures from the museum of the Department of Biochemistry, Microbiology and Nourishment Physiology at Odessa National Academy of Food Technologies, Ukraine, *Bifidobacterium longum-Ya3, Bifidobacterium adolescentis-C52, Propionibacterium shermanii-PS4*.

As the examined samples we employed the leaven of monoculture and symbiotic consortium of bifido- and propionic acid bacteria. As a control we applied the composition of fatty acids (FA) of nutrient medium without introducing the culture (Fig. 1). The cultivation of symbiotic leaven was conducted in the ratio 1:1 (*B.longum- Ya3:P.shermanii-PS4; B.adolescentis-C52:P.shermanii-PS4*) on the lactose medium with the addition of soybean serum at temperature (34±1) °C during 24 h. The following components are included in the composition of medium:

1) agar-agar 0.25 % by weight;
2) peptone 0.1 % by weight;
3) soybean serum 3 % by weight;
4) sodium citric-acid trisubstituted 0.6 % by weight;
5) potassium phosphoric acid disubstituted 0.2 % by weight;
6) magnesium sulphate 0.12 % by weight;
7) lactose 0.1 % by weight;
8) ascorbic acid 0.05 % by weight.

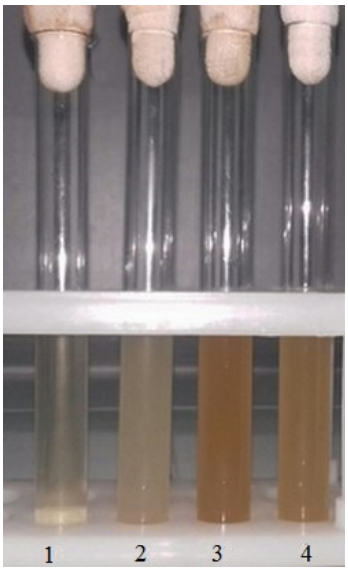

Fig. 1. Experimental samples: 1 – control; 2 – *P.shermanii-PS4*; 3 *B.longum-Ya 3:P.shermanii-PS4*; 4 – *B.adolescentis-C52:P.shermanii-PS4*

We poured distilled water into the mixture thus bringing the volume to 100 % by weight. The active acidity of medium (7±0.1) pH units was established using the 30 % solution of NaOH or the 25 % solution of ammonia [13, 14].

Accounting for the titre of diurnal cultures was carried out by the direct calculation of propionic acid bacteria in the counting chambers of Goryaev and by sowing the bifidobacteria into semi-fluid nutrient medium.

The activity of acid formation of the examined samples was determined by the titrometric method. We calculated the titrated acidity by formula:

$$°T = A \times K \times 10, \quad (1)$$

where A is the amount of milliliters 0.1 M, which was used for the titration of 10 cm^3 of the culture fluid; K is the amendment to the titer 0.1 M of the solution of sodium hy-

droxide; °T is the conditional magnitude, expressed in the Turner's degrees.

4. 2. Methods of experimental studies, utilized when conducting the study

When conducting the studies, we used the method of determining the total quantity of protein in the biomass of bacteria, which is described in [15], of carbohydrates – according to [16]; the total amount of nuclein acids in the biomass was determined by method [17]; of mineral substances – by [18].

4. 3. Procedure of determining the amount of fatty acids in the summary fraction of lipids

Lipids were extracted from 1 g of mass of the material dried lyophillically, which was examined at reduced temperatures first by isopropynol, next by isopropanol-chlorine (1:1) and twice by the mixture of chloroform-methanol (1:1). The amount of fatty acids (FA) in the summary fraction of lipids was determined using the gas-liquid chromatography in the form of methyl ethers.

An analysis of methyl ethers of fatty acids was carried out by the method of gas-liquid chromatography using the gas chromatograph GC-17A "Shimadzu" (Japan) with the possibility of programming the temperature to 330 °C, by the flame-ionization detector and the software "GC solution" (Japan). For the separation we used the capillary column THERMO TR-FAME (Germany) (30 mm×0.25 mm ID×0.25 um film) with a temperature gradient from 70 °C to 230 °C (Fig. 2).

Fig. 2. Gas chromatograph GC-17A "Shimadzu" with the software "GC solution", (Japan)

Stationary phase is 70 % Cyanopropyl (equip) Polysiphenylene-siloxane. Non-stationary phase is helium, gas flow rate is 1 ml/min. The temperature of injector and detector was equal to 280 °C and 260 °C, respectively. The content of FA was expressed in percent of the total sum. The identification of FA was carried out by comparing the time of holding the determined compounds to the time of holding the standard FA [19].

5. Results of examining the fatty acid composition of probiotic consortia with the inclusion of propionic acid bacteria

Results of the studies demonstrated that the propionic acid bacteria in the association with bifidobacteria have the higher biochemical activity than that in a monoculture. This is evidenced by both an increase in the biomass of cells of bifido- and propionic acid bacteria and an increase in the acidic potential of the combined leaven (Table 1).

Table 1

Development indicators of the examined bacteria in a monoculture and in the consortium (n=3, P≤0.95)

Indicators	In a monoculture	In the consortium of bacteria at ratio 1:1	
	P.shermani-PS4	*B.longum-Ya* 3+ *P.shermanii-PS4*	*B.adolescentis-C52+P.shermanii-PS4*
Quantity of cells of bifidobacteria, KOE/cm^3	–	$3 \cdot 10^{10}$	$2 \cdot 10^{10}$
Quantity of cells of the propionic acid bacteria, KOE/cm^3	$2 \cdot 10^9$	$4 \cdot 10^{10}$	$2 \cdot 10^{10}$
Active acidity, pH	5,9	5.4	5.3
Titrated acidity, °T	70	74	73

Density of the population of probiotic microorganisms in the consortium increased from 10^9 KOE/cm^3 to 10^{10} KOE/cm^3, which indicates the existence of symbiotic bonds of bifido- and propionic acid bacteria. Probably, the carbohydrates of propionic acid bacteria stimulate the growth of bifidobacteria. Such conclusions confirm both the high quantity of viable cells of both types in the consortium – Lg $2\text{-}4 \cdot 10^{10}$ KOE/cm^3 and the content of carbohydrates in the biomass of the examined bacteria in the monoculture and in the consortium (Table 2). We should note the higher synthesis of carbohydrates in propionibacteria whose content made up 23.39 %. However, at combined cultivation the amount of carbohydrates decreased approximately to 16 %. It should be noted that in the consortium of *B.longum- Ya* 3+*P.shermanii-PS4* and *B.adolescentis-C52+ +P.shermanii-PS4,* the content of carbohydrates was higher than in the monoculture of bifidobacteria, and it amounted to 18.93 % and 18.86 %, respectively.

The present study deals with the research into the content of general lipids and their fatty acid composition both in a monoculture and in the consortium of bifido- and propionic acid bacteria.

An analysis of composition of fatty acids in the general fraction of lipids revealed that the unsaturated FA prevailed over those saturated in all examined models (Fig. 1). Nutrient medium was prepared using a soybean serum, which explains the fact that the fraction of PUFA in the control was larger than that in the examined samples and made up 28.80 % of the sum of FA.

After the introduction and cultivation of the examined samples, the amount of PUFA varied in the range of 3.96–23.99 % of the sum of FA. The fraction of PUFA was absent from the sample of leaven with the use of *B.adolescentis-C52+P.shermanii PS4*. This proves the capability of the examined microorganisms to convert linoleic acid.

In all the examined leaven, the fraction of MNF**A** predominated, its percentage was within the range from 54.26 to 68.42 % of the sum of FA, which is 1.5–2 times is larger than the values of the control. The value of control was the following – 9.53 %.

Table 2

Chemical composition of the biomass of bacteria in a monoculture and in the consortium (n=3, P≤0,95)

Sample, No.	Mineral substances, %	Protein, %	Lipids, %	Nuclein acids, %	Carbohydrates, %
B.longum- Ya3+ P.shermanii-PS4	4.94	39.76	18.48	17.89	18.93
B.adolescentis-C52+ P.shermani-PS4	6.98	38.82	18.48	16.86	18.86
P.shermani-PS4	6.4	33.21	19.21	17.79	23.39
B.longum-Ya3	7.89	37.43	18.98	19.75	15.95
B.adolescentis-C52	7.99	37.42	18.88	19.68	15.93

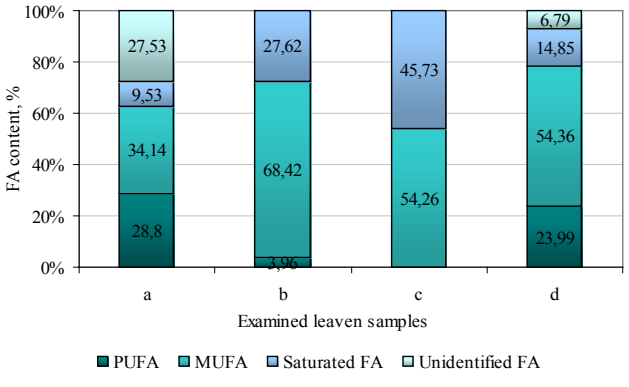

Fig. 3. Total content of FA in the samples of leaven (% by weight): *a* – control sample, *b* – *P.shermanii-PS4*, *c* – *B.adolescentis-C52* + *P. shermanii-PS4*, *d* – *B. longum-Ya 3*+*P.shermanii-PS4*

The saturated acids made up from 14.85 to 27.62 % of the sum of FA. The highest content of saturated FA was demonstrated by sample of the leaven *B.adolescentis-C52+P.shermanii-PS4* and it amounted to 45.73 % of the sum of FA.

Results of examining the composition of FA leaven *P.shermanii-PS4* are given in Table 3. Dominating FA are palmitic acid (C16:0) in the amount of 20.88 % of the sum of FA, oleic acid (C18:1) – 68.42, while the amount of linoleic acid (C18:2n-6) was minimum – 3.96 %.

Palmitic acid (C16:0) and oleic acid (C18:1) prevailed in the composition of the leaven *B.adolescentis-C52+P.shermanii-PS4*. The amount of palmitic acid made up 20.67 % of the sum of FA. The content of oleic acid in the given leaven was the largest – 54.26 %. However, linoleic acid (C18:2n-6) was not detected at all (Table 4).

It should be noted that among the examined samples, the leaven *B.longum-Ya3+P.shermanii-PS4* contained the maximum amount of linoleic acid (C18:2n-6) – 23.99 % of the total quantity of FA. Dominating FA is oleic acid (C18:1) in the amount of 54.36 (Table 5).

It is evident from the results of experimental data that practically in all samples the basic FA are C16:0, C18:1. The most valuable composition of FA was demonstrated by leaven from the symbiotic consortium *B.longum-Ya3* and *P.shermanii-PS4*. In this model, the sum of unsaturated fatty acids (78.36 %), was larger than in other examined samples. The amount of linoleic acid made up 23.99 %. Furthermore, the probiotic leaven of the consortium *B.longum-Ya3* and *P.shermanii-PS4* was characterized by the largest indicators of biochemical activity.

Table 3

Fatty acid composition of sample of the leaven *P.shermanii-PS4*

Time, min	Component	Group	Area	Height	Concentration, %
12.940	C16:0 Palmitic	Saturated	6021	24009	20.879
14.755	C18:0 Stearic	Saturated	1944	617	6.742
14.942	C18:1 Oleic	Monoun-saturated	19731	926	68.419
15.620	C18:2 Linoleic	Polyun-saturated	1142	339	3.961
TOTAL CONTENT OF FA					
Total saturated FA			27.62 %		
Total unsaturated FA			72.38 %		

Table 4

Fatty acid composition of sample of the leaven *B.adolescentis-C52+P.shermanii-PS4*

Time, min	Component	Group	Area	Height	Concentration, %
3.434	C6:0 Kapron	Saturated	308	99	5.454
10.429	C14:0 Myristic	Saturated	285	125	5.051
12.938	C16:0 Palmitic	Saturated	1168	473	20.676
14.752	C18:0 Stearic	Monoun-saturated	413	125	7.30
14.930	C18:0 Stearic	Monoun-saturated	410	77	7.259
15.062	C18:1 Oleic	Monoun-saturated	3066	886	54.260
TOTAL CONTENT OF FA					
Total saturated FA			45.73 %		
Total unsaturated FA			54.26 %		

Table 5

Fatty acid composition of sample of the leaven *B.longum- Ya3+P.shermanii-PS4*

Time, min	Component	Group	Area	Height	Concentration, %
12.945	C16:0 Palmitic	Saturated	1367	561	9.958
14.761	C18:0 Stearic	Saturated	672	213	4.895
14.945	C18:1 Oleic	Monoun-saturated	1116	210	8.132
15.071	C18:1 Oleic	Monoun-saturated	6346	2007	46.227
15.630	C18:2 Linoleic	Polyun-saturated	3294	1123	23.997
22.503	Unidentified FA		932	180	6.791
TOTAL CONTENT OF FA					
Total saturated FA					14.85 %
Total unsaturated FA					78.36 %
Others					6.79 %

6. Discussion of results of examining the fatty acid composition of probiotic consortia with the inclusion of propionic acid bacteria

Given the development of industrial market for biologically active additives (BAD) that contain PUFA, there emerged the need to search for new sources. The promising source of lipids, namely PUFA, are microorganisms. They have a number of advantages compared with the raw materials of plant and animal origin. Microorganisms are capable of rapidly accumulating the biomass on simple media. In this case, they synthesize and accumulate in cells up to 50 % of PUFA [20]. It should be emphasized that in the course of present research an analysis of the fatty acid composition of probiotic bacterial leaven in order to creat BAD was carried out for the first time. Results of the study might be used in the field of biotechnology, microbiology, medicine and cattle breeding.

After the cultivation of probiotic cultures on the soybean-lactose medium for 24 hours at temperature (34±1) °C, the quantity of bacteria reached Lg $2–4 \cdot 10^{10}$ KOE/cm^3. Such quantity of cells is large enough. The most valuable composition of FA was demonstrated by the sample *B.longum-Ya3* and *P.shermanii-PS4*. In this probiotic ferment the amount of PUFA was larger than in the remaining samples and made up 23.9 % of the total quantity of FA.

In order to increase the content of practically important essential PUFA in the probiotic consortia, it is planned to conduct the selection of cultivation conditions.

7. Conclusions

Obtained data indicate the prospect of creating the biologically active additives and functional food products based the combined cultivation of bifidobacteria and propionic acid bacteria with the increased content of essential fatty acids.

1. In the course of examining the accumulation of biomass of the examined strains, *Bifidobacterium longum-Ya3, Bifidobacterium adolescentis-C 52, Propionibacterium shermanii-PS*, it was found that there exists a symbiotic bond between the propionic acid bacteria and bifidobacteria. Upon the cultivation of cultures for 24 hours at temperature (34±1) °C, density of the population of probiotic microorganisms in the consortium increased from 10^9 KOE/cm^3 to 10^{10} KOE/cm^3. In this case, the biochemical activity of the combined leaven exceeded that in a monoculture.

2. It was established that *B.longum-Ya3* and *P.shermanii-PS4*, cultivated on the medium with the addition of soybean serum in the amount of 3 %, possessed pronounced synthetic activity relative to the polyunsaturated fatty acids, using oleic acid as the predecessor.

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