Qualitative Characteristics of Bacterial Concentrate of Microbial Consortium

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ABSTRACT
The article presents the results of technological development to receive bacterial concentrate of the microbial consortium that can be applied in fermented milk production of mixed fermentation and as a biologically active food additive. It was investigated the possibility of using rye flour to increase the biomass of microbial consortium, which has been obtained by auto-selection of kefir starter and thermophilic lactobactillus microflora. The qualitative characteristics of bacterial concentrate indicate its high biochemical activity.

Keywords*: *microbial consortium, bacterial concentrate, biologically active food

1. INTRODUCTION

Many researchers support the view that the endoecology of human and animals serves as nutritional homeostasis that destructs food components and forms deficient products. With that in mind, it necessary to consume products that provide colonization of the gastrointestinal tract by probiotic microorganisms.

The microflora of lactic acid products of heterofermentative fermentation is a symbiotic community of microorganisms. The interest in natural symbiotes to create probiotics is caused by the fact that after the interruption of supporting therapy by strain preparations, the latter stop was progressing and replace by microflora. Symbiotic multi-component of the lactobacteria microflora organizes a strong link cluster and sustainable bioresonance system of endosymbioses that provide the sustained results [6].

2. THE PURPOSE OF THE STUDY

The main objective of the work is to study the quantitative characteristics of bacterial concentrate of the microbial consortium.

3. THE OBJECT OF THE STUDY

The object of the research is a bacterial concentrate of microbial consortium produced by auto-selection of kefir grain microflora with thermophilic lactobacillus L.acidophilus, L.bulgaricus, L.helveticus in ratio 1:0,5:0,5:1 [3]

4. MATERIAL AND METHODS

In work were used standard methods of physical, chemical and microbiological indicators:

- Biomass growth – measuring optical density by photocolorimetric method on KF-77 = 550 nm;
- The quantitative account of lactose and lactose-free yeast was conducted using the method of limiting dilutions by the number of colony-forming units on lactose and glucose potato agar, lactobacteria on hydrolyzed milk medium;
- The microorganism cells affect properties on the adhesion process was studied using the Brill method [2];
- The antibiotic activity was determined using the Polonsky's method of sequential. In the research of antibiotical activity was used the culture test *E. coli* I and *S. sonnei* 2848.

5. DISCUSSION OF THE RESULTS

Microbial consortium contains a variety type of lactobacteria, yeast, and acetobacter. For the balanced growth of microorganisms, it needs a wide range of carbohydrates. Therefore, it should be used nutrient medium containing complex. The nutrient medium should consist of a cheap renewable substrate, such as grain flour. For the fermentation starter of lactobacteria culture and yeast was established the technology of rye bread production. The pregelatinized rye flour includes *Lactobacillus plantarum, L.brevis, L. delbrueckii,*

L.fermentum, *Saccharomyces cerevisiae*, and *S. minor*. The rye flour contains carbohydrates such as maltose, galactose, glucose, sucrose, and dietary fibre, which have a significant proportion of water-soluble proteins. The mineral composition of rye flour contains phosphorus, potassium, magnesium, and calcium [4].

In this regard, the first stage of research is to investigate the effect of rye flour doze on the microorganisms of the microbial consortium. As the basis of the nutrient medium, serum was used with the addition of unpolished flour, and the sample without adding rye flour was used as a control, the dynamics of biomass growth were estimated by optical density.

The research results show that adding the rye flour to the nutrient medium increases the growth of microorganisms which account for microbial consortium.

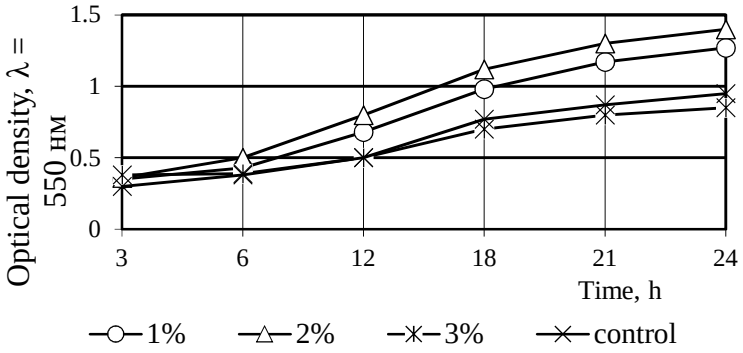
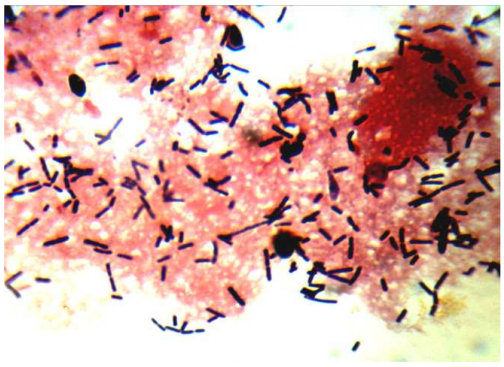
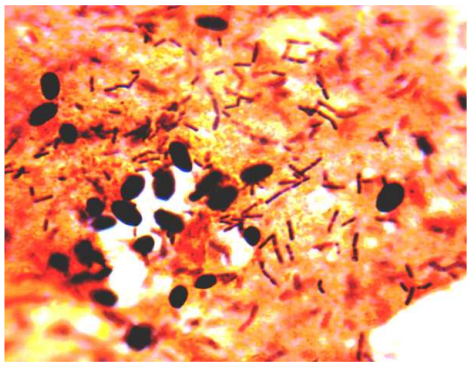


Figure 1 Dynamics of inoculated nutrient media optical density

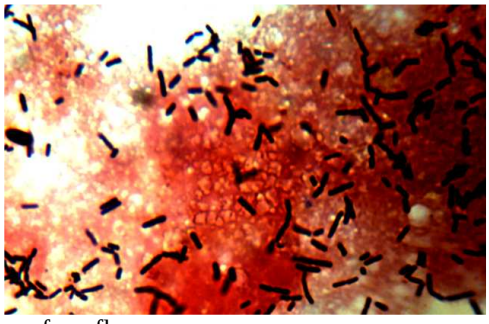
It was found that the addition of 2% rye flour provides an increase of mesophilic lactobacteria up to $4 \cdot 10^{11}$ CFU/mL in 24 hours, thermophilic lactobacteria up to $5 \cdot 10^{10}$ CFU/mL and yeast, non-fermenting lactose up to $3 \cdot 10^{8}$ CFU/mL .The amount of yeast fermenting lactose increases up to $1 \cdot 10^{7}$ CFU/mL. The resulting ratio of microorganisms groups complies with an initial ratio in the microbial consortium which was received by auto-selection of microflora of the kefir yeast and thermophilic lactobacteria. An increase of introduced rye flour doze up to 3 % provides the maximum viable cells up to $3 \cdot 10^{9}$ CFU/mL. A decreased growth of microorganisms related to the colloidal properties of the rye flour in adding 3 % of rye flour, which reduces the amount of free water, which is vital for bacteria. Figure 2 presents the results of dosing rye flour introduced into the nutrient medium on the morphology of microbial colonies of the microbial consortium.



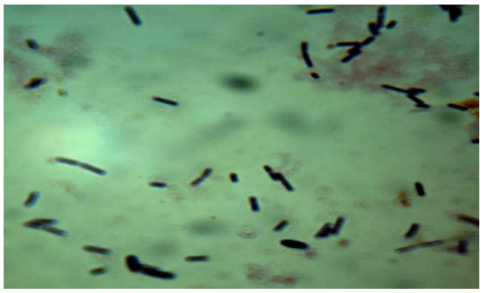
with 1 % doze of rye flour



b) with 2 % doze of rye flour



with 3 % doze of rye flour



d) with 2 % doze of rye flour

Figure 2 The effect of the rye flour doze on the micro picture of the samples.

Figure 2 shows the micro picture of yeast cells samples presented by thin sticks of different lengths. The most massive accumulation of large yeast cells and lactobacteria was observed in the sample with a dose of rye flour 2 % (Fig. 2, b). The formation of microbial matrices facilitates the rapid exchange of vital activity, protects bacteria from negative consequences of environmental conditions. In nature, natural populations of microorganisms prefer to grow on solid surfaces in an attached state. The mucous secretions of these cells contribute to the attachment to a solid substrate, i.e. adhesion of microorganisms on solid surfaces is a vital way of adaptation to various habitats, and it determines their stability and protective properties [4, 5].

Adhesion properties were evaluated in the biomass obtained on a nutrient medium with 2 % rye flour, as a control used the image without the addition of rye flour.

Table 1 shows that the introduction of rye flour increases the adhesive properties of the microbial consortium. Thus, the adhesiveness index in a sample with 2 % rye flour exceeds this control indicator on 0,6.

Table 1 Adhesive properties of the microbial consortium

Indicator	**Name of experience**	
	Doze 2 %	**Control**
Average indicator of adhesion	4,6	3,2
The coefficient of erythrocytes participation in the adhesion process, %	85	79
Adhesion index of microorganisms	5,4+1,1	4,0+1,1
Adhesion properties	Highly adhesive	Medium adhesive

Further, the antagonistic activity was studied in obtained bacterial concentrate. The antagonistic activity of microbial consortium was determined the method of sequential breeding by the size of inhibition zones of the culture test *E. coli* I_{53} and *S. sonnei* 2848 with regard to pathogenic conditionally pathogenic microorganisms. The results of the study are presented in Table 2.

Table 2 Antagonistic activity of microbial consortium and kefir grain

Types of a starter culture	**Bacteria growth in dilution**			
	***E.coli* I_{53}**		***S.sonnei* 2848**	
	no growth	**growth inhibition**	**no growth**	**growth inhibition**
Kefir grain	1:4	1:32	1:4	1:64
Microbial consortium	1:8	1:64	1:16	1:128

Table 2 shows that the bactericidal effect of the microbial consortium concerning *E. coli* was manifested in a dilution of 1: 8, and in *S. sonnei* – 1:16. The bacteriostatic effect of these starter cultures was noted at dilutions of 1 : 64 and 1: 128, respectively.

Table 3 The qualitative characteristics of the bacterial concentrate

Indicator	The value of the indicator
Consistency and appearance	Homogeneous liquid. Separation is allowed
Colour	From white to light brown, with dark inclusions
Taste and smell	Lactic acid with a touch of rye flour
Mass fraction of solids,%	7,2±0,5
Active acidity (pH)	5–7
Fermentation temperature ° C	28±2
Activity (fermentation duration 10 dm^3 when the concentrate is added with 1 activity unit)	10–12
Titratable acidity ° T	120
Release temperature 0 ° C	4±2
Duration of storage, month	3
Number of microorganisms:	
Lactobacteria:	
thermophilic	$5 \cdot 10^{10}$
mesophilic	$4 \cdot 10^{11}$
Yeast:	
non-lactose-fermenting	$3 \cdot 10^{8}$
lactose-fermenting	$2 \cdot 10^{7}$
Micropicture	single thin sticks of different lengths, single and clusters of yeast
The volume of concentrate in cm^3, in which there are no	
Coliform bacteria	1,0
S. aureus	1,0
Pathogenic, including Salmonella	10,0

The data indicate that the microbial consortium is able to suppress the growth of pathogenic and opportunistic microflora.

The qualitative characteristics of the bacterial concentrate of the microbial consortium obtained in a nutrient medium with 2 % rye flour are presented in Table 3.

From Table 3, we can conclude that the produced liquid bacterial concentrate makes it possible to use the direct method of producing fermented milk products of mixed fermentation (koumiss and kurunga) and as a biologically active food additive. This result is because bacterial concentrate is characterized by a high concentration of viable cells and fermenting activity.

6. CONCLUSION

It is proved that the introduction of 2% rye flour into the nutrient medium provide a bacterial concentrate with a high content of viable cells of the microbial consortium: mesophilic lactobacilli up to $5*10^{11}$ CFU/ml and yeast that does not ferment lactose – up to $3 \cdot 10^{8}$ CFU / ml. At the same time, the initial microbial consortium retains the ratio of microorganisms in the bacterial concentrate. It was also found that the presence of the rye flour in a nutrient medium contributes to an increase in the adhesive properties of microorganisms. The result of bacterial concentrate has antagonistic activity against the pathogenic and conditionally pathogenic microflora.

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