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Influence of Dairy Microorganisms and their Consortia on Indigenous Microflora

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ABSTRACT
The influence of consortia of dairy lactic acid bacteria, acetic acid bacteria, propionibacteria and lactose-fermenting yeast on the growth of indigenous lactobacilli isolated from feces of healthy people and probiotic preparations was studied. The effect of lactic acid bacteria on lacto- and bifidoflora depends on the degree of their antagonistic activity. Bacteriocins-producing lactic acid bacteria show antagonism against indigenous lactic microflora. Lactose-fermenting yeast and acetic acid bacteria stimulated growth of indigenous lactic acid bacteria. Inhibitory effect of lactic acid bacteria and their consortia with yeast, propionibacteria and acetic acid bacteria on *Bifidobacterium bifidum* can be reduced or completely eliminated up to growth stimulation by introducing into the culture medium a prebiotic additive in the form of wheat bran.
Keywords – antagonism, consortium, dairy microorganisms, indigenous microflora, stimulation.

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I. INTRODUCTION

Currently, the intestinal microbiota is considered to be the main determinant of human health and disease. There are evidences that a change in the relationship between the composition of the intestinal microbiota and the human body is accompanied by the development of allergic and immunopathological conditions, as well as a number of diseases such as obesity, type II diabetes, inflammatory bowel disease, irritable bowel syndrome, various types of cancer and others [1, 2]. Consequently, the manipulation of the gut microbiota can be employed to prevent or treat these conditions [3]. The most prevalent strategy to correct intestinal microbiota is the administration of probiotics [4].

The use of probiotics and synbiotics has shown promising results against various intestinal pathogens due to the unique ability of their microorganisms to compete with pathogenic microbiota for adhesion sites, suppress pathogens, and regulate the host immune response [5].

However, the safety and dosing concerns continue to temper widespread use of probiotics [6]. The administration of probiotic strains may not always be safe and effective and probiotic effect may not always be achieved [7-9]. The strains shown to confer a benefit for one condition may not be probiotic for another application [10]. The available data on some effects of probiotics are incomplete and far from conclusive [11]. A few reports about negative probiotic effects have surfaced [12-13]. The use of probiotic or synbiotic preparations in critically ill patients continues to be controversial [14].

There is evidence that probiotic lactobacilli are capable of causing an imbalance in the native host lactoflora. Thus, in the study of the relationship of five industrial probiotic strains of lactobacilli and 458 cultures of indigenous lactobacilli isolated from the human digestive and vaginal tracts, as well as 98 isolates of white rat and mouse feces, it was shown that probiotic strains inhibited in vitro more than 60% of the cultures of indigenous lactobacilli [15].

Analysis of the reasons for not always convincing clinical use of probiotics leads to the conclusion that many factors can influence the survival and activity of probiotic strains in the host organism, among which antagonism between probiotic bacteria and the resident microflora may have a significant value [16].

More studies are required to accurately define the occurrence and severity of unfavorable events linked to probiotics. And more complete understanding of the mechanisms of probiotic interaction with the host and colonizing microbes is necessary [4, 12, 17].

The purpose of this work was to study the effect of potentially probiotic lactic acid bacteria and their consortia with lactose-fermenting yeast, acetic acid bacteria and propionic acid bacteria on indigenous microorganisms.

II. MATERIALS AND METHODS

The objects of investigation were lactic acid bacteria: *Lactobacillus delbrueckii* 5, *L. gallinarum* 1, *L. paracasei* 33-4, *L. parabuchneri* 3, *L. fermentum*, possessing high antagonistic activity, as well as 29 isolates of lactic acid bacteria from camel, mare and goat milk; acetic acid bacteria *Acetobacter indonesiensis* 2; propionic bacteria *Propionibacterium freudenreichii subsp. shermanii*; lactose-fermenting yeast *Kluyveromyces marxianus* 19 and *K. marxianus* Dkum5(30)2.

Representatives of the indigenous intestinal microflora were isolated from feces of healthy people and probiotic preparations Linex (Russia) - *Lactobacillus acidophilus*, *Enterococcus faecium*; Maxilin (Kazakhstan) – *L. acidophilus*; weight loss biopreparation (China) - *L. acidophilus*; Bifidumbacterin (Russia) - *Bifidobacterium bifidum*; Kolibakterin (Russia) - *Ecsherichia coli*. Probiotic yeasts were also obtained from Enterol (Russia) - *Saccharomyces boulardii*. Molecular identification of intestinal microflora was carried out by the method of sequencing 16S rRNA by Sanger.

Lactic acid bacteria were cultivated in MRS (de Man, Rogosa and Sharpe) medium (TM Media, India) for 2 days at 37° C. Bifidobacteria were cultured in Bifidobacterium agar (TM Media, India), or in MRS in combination with anaerobic agar (TM Media, India) in a ratio of 1:1, or in the Blaurock medium, g/l: peptone 10.0; yeast extract 0.5; lactose 10.0; sodium chloride 5.0; magnesium chloride 5.0; cysteine hydrochloric acid 1.0; agar-agar 0.75; tween-80 1.0 ml; distilled water 1,000 ml; pH 7.2. Test cultures of *E. coli* were grown on Nutrient Agar medium (TM Media, India).

The effect of microorganisms of the consortia on the indigenous microflora was investigated by the well method. For this, individual cultures and consortia were cultivated in cow's milk with 1.5% fat (Lactel, Food Master, Kazakhstan) for 16–20 hours, as well as in milk with the addition of 1-2% wheat or oat bran. 0.3 ml of fermented milk was added to each well (10 mm in diameter) in the lawn of a test culture. Plates with bifidobacteria were placed in a desiccator with a lit candle and greased with vaseline edges to reduce the access of oxygen. All plates were incubated at 37° C for 2-3 days. The effect on test cultures was judged by the presence and size of inhibition or stimulation zones around the wells.

All experiments were performed in triplicate. Statistical processing of research results produced by the standard method using Student's t-test. The P-value was <0.05.

III. RESULTS AND DISCUSSION

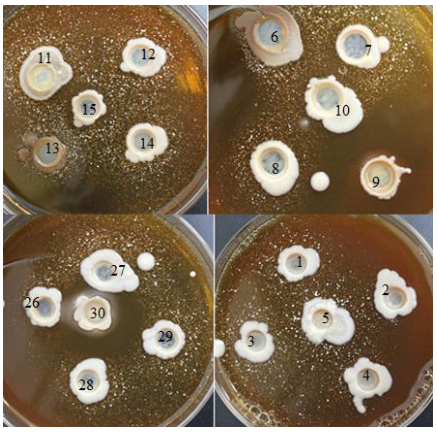
To determine the effect of dairy microorganisms on intestinal autoflora, the main representatives of indigenous human gut microbiota were isolated from feces of healthy people and probiotic preparations. Lactic acid bacteria isolated from feces were identified as *Enterococcus durans* and *L. pontis*. The degree of homology with the closest strains was respectively 100% and 98.71%.

In the study of the effects of lactic acid bacteria on indigenous lactic acid bacteria isolated from feces and probiotic preparations, it was found that the antagonistically active bacteriocin-producing lactobacilli *L. delbrueckii* 5, *L. gallinarum* 1, *L. paracasei* 33-4, *L. parabuchneri* 3 and *L. fermentum* suppress indigenous lactobacilli and enterococci. Growth suppression of *L. acidophilus*, however, was not complete when growing microorganisms on cow's milk without additives.

Isolates of lactic acid bacteria not producing bacteriocins (not suppressing a wide range of bacterial test cultures), did not affect indigenous lactic acid bacteria as well.

Figure 1 shows the effect of the associations of various isolates of lactic acid bacteria with the yeast *K. marxianus* 19 on the heterofermentative lactic acid bacteria *L. pontis* isolated from feces.

Associations of lactic acid bacteria with yeast exerted various effects on *L. pontis* depending on the presence of pronounced antibacterial activity in lactic acid bacteria. Thus, the association number 2 did not affect the growth of the test culture of lactic acid bacteria; associations No. 9 and No. 30 inhibited the growth of *L. pontis*; associations Nos. 7, 10 and 15 slightly inhibited the growth of lactobacilli in the vicinity of the well, showing a stimulating effect outside this zone; the remaining associations significantly stimulated the growth of lactic acid bacteria. The stimulating effect was expressed both in the appearance of colonies on the surface of the medium and in the increase in their



1-15, 26-30 - associations of various isolates of lactic acid bacteria with lactose-fermenting yeast
K. marxianus 19

Figure 1 - The effect of lactic acid bacteria and lactose-fermenting yeast associations on *L. pontis*

The impact of lactic acid bacteria on B. bifidum bifidobacteria was ambiguous. When conducting research in a mixture of MRS medium with anaerobic agar, a slight stimulation of the growth of bifidobacteria was detected by various strains of lactic acid bacteria. Interestingly, when conducting an experiment on a favorable environment for bifidobacteria (Bifidobacterium agar), all antagonistically active lactic acid bacteria inhibited the growth of *B. bifidum*.

The effect of lactic acid bacteria on *E. coli* was dual. The growth of *E. coli* was partially suppressed in the immediate vicinity of the well, and was stimulated outside this zone.

Lactose-fermenting yeast stimulated the growth of lactic acid bacteria *L. acidophilus* from the Linex preparation and did not affect the same species isolated from other probiotics.

Yeast *K. marxianus* stimulated the growth of all indigenous lactic acid bacteria.The stimulation of microorganisms of *Enterococcus* genus was especially pronounced (Fig. 2).

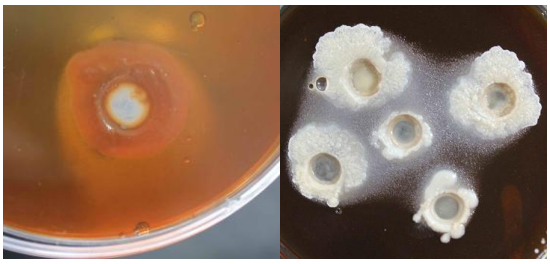


Figure 2 - Effect of *K. marxianus* 19 on *E. faecium* (left) and *E. durans* (right)

In mixed culture of lactose-fermenting yeast and not producing bacteriocins lactic acid bacteria, the zones of stimulation enterococci growth coalesced into a solid dense layer on the surface of the medium, although these microorganisms did not grow in the control on the surface of agar.

K. marxianus did not affect bifidobacteria and *E. coli* growth.

Propionic bacteria P. *freudenreichii subsp. shermanii* stimulated the growth of L. acidophilus from the probiotic Maxilin, without affecting *L. acidophilus* from other drugs, and only slightly suppressed the growth of *E. coli*, stimulating it to some extent outside the zone of suppression. Propionic bacteria showed a stimulating effect on the culture of probiotic yeast *S. boulardii*.

Acetic acid bacteria stimulated to some extent the growth of all indigenous lactic acid bacteria.

The possibility of increasing the normoflora stimulating activity of lactic acid bacteria by introducing prebiotic supplements in the form of oat and wheat bran, as well as creating consortia of dairy microorganisms of different taxonomic position, has been investigated.

The influence of the consortium No. 1 (*L. delbrueckii* 5, *L. gallinarum* 1, *L. paracasei* 33-4, *L. parabuchneri* 3, *A. indonesiensis* 2, and *K. marxianus* 19) and No. 15 (*L. delbrueckii* 5, *L. gallinarum* 1, *L. paracasei* 33-4, *L. parabuchneri* 3, *A. indonesiensis* 2, *P. freudenreichii subsp. shermanii*, *K. marxianus* 19, and *K. marxianus* Dkum5(30)2) and their lactic acid bacteria on the growth of *B. bifidum* when cultured on milk without additives and milk with the addition of wheat or oat bran was studied.

The dependence of the effect of lactic acid bacteria on bifidobacteria on the medium of experiment conducting was revealed.

Cultivation of pure cultures of lactic acid bacteria of the consortia in milk with addition of 1% oat bran showed slightly lower stimulating effect on bifidobacteria comparing with the variants without bran in the experiment with MRS medium and anaerobic agar. Growing microorganisms in milk with the addition of 1% wheat bran did not change the degree of B. bifidum stimulation by pure cultures of lactic acid bacteria, but increased bifidoflora stimulating activity of consortia by 23-31%.

When conducting experiment in Bifidobacterium agar, both consortia cultured in milk without additives inhibited the growth of bifidobacteria. The addition of 1% wheat bran decreased inhibition *B. bifidum* zones by 10-25% by three of six studied cultures compared with milk without bran.

Growing the consortia in milk with the addition of 2% oat bran reduced the growth inhibition of *B. bifidum* by consortium No. 1 by 12-20% and completely removed the inhibitory effect of the consortium No. 15. Stimulation of *B. bifidum* growth in the vicinity of the wells was also noted for consortium No. 15 (Fig. 3).

Thus, despite the suppression of the growth of indigenous microflora by antagonistically active lactic acid bacteria, the inhibitory effect can be reduced or completely eliminated up to growth stimulation by introducing into the culture medium a

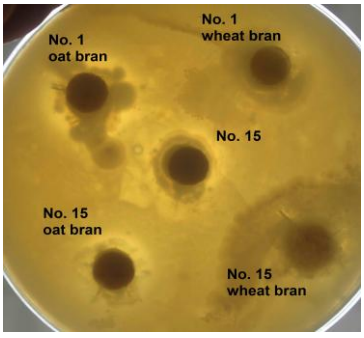


Figure 3 - Effect of consortia No. 1 and No. 15 in milk with 2% oat and wheat bran on *B. bifidum* in Bifidobacterium agar

prebiotic additive in the form of wheat bran. The change in influence of lactic acid bacteria on indigenous microflora when cultivated in an environment with wheat bran may be due to the spatial separation of indigenous and probiotic microorganisms following cell adhesion on bran, as well as owing to the influence of bran on the metabolites production of lactic acid bacteria. Control milk with bran did not show any stimulating effect on bifidobacteria.

IV. CONCLUSION

Bacteriocin-producing lactic acid bacteria are most likely to show antagonism against members of the indigenous lactic microflora. Lactose-fermenting yeast promotes the growth of lactic microflora, showing a particularly pronounced effect on the growth of enterococci. Acetic acid bacteria stimulate the growth of all lactic acid bacteria to some extent. Propionic acid bacteria showed stimulating effect only on probiotic *S. boulardii* yeast. The influence of lactic acid bacteria on bifidobacteria can depend on the environment of the experiment.

The combination of the consortium of microorganisms with different types of metabolism, namely antagonistically active lactic acid bacteria, lactose-fermenting yeast, acetic acid bacteria and propionibacteria, with the additional administration of plant fibers in the form of wheat bran has beneficial effect on the growth of *B. bifidum*, which is the main representative of the indigenous microflora of thick intestine.

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