PROBIOTIC CONSORTIUMS: STRUCTURE AND ANTAGONISTIC ACTIVITY AGAINST OPPORTUNISTIC BACTERIA AND HUMAN NORMOBIOTA (USING THE EXAMPLE OF *ESCHERICHIA COLI*) *IN VITRO*

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ABSTRACT

Background. *Using probiotic preparations based on consortia of microorganisms not only helps to restore the balance of the intestinal microbiota, but also increases the therapeutic effect of probiotics. Promising sources for obtaining probiotic consortia are milk products that have undergone natural fermentation with the help of spontaneously formed microbial consortia.*

The aim. *To study the structure of five microbial consortia with probiotic properties from naturally fermented milk products and to assess in vitro their antagonistic activity against opportunistic bacteria and a representative of the human normobiota – Escherichia coli.*

Materials and methods. *The structure of bacterial consortia was analyzed by sequencing methods. The antagonistic activity of the consortia was assessed by the disk diffusion method.*

Results. *It has been established that the studied microbial consortiums are represented by Enterococcus spp. and Streptococcus spp. bacteria. In consortiums No. 1, No. 2, and No. 3, Enterococcus bacteria dominated, while in consortiums No. 4 and No. 5, Streptococcus dominated. Antagonistic activity was shown against four isolates of opportunistic bacteria: Klebsiella pneumoniae No. 493, Enterobacter hormaechei No. 372, Staphylococcus aureus No. 4 and Pseudomonas aeruginosa No. 25 IMB, as well as against one representative of the human normobiota – Escherichia coli No. 495. The highest growth delay zone is found in E. coli No. 495 isolate. Three test cultures (K. pneumoniae No. 509, E. coli ATCC25922 and P. aeruginosa No. 3 IMB) exhibited more dense growth around probiotic consortia.*

Conclusion. *The results of the study showed that the effect of probiotic consortia differing in the composition of microorganisms can be neutral and bactericidal. The presence of antagonistic activity in the studied microbial consortia against multiresistant isolates of opportunistic bacteria is a prospect for creating probiotics with antibacterial properties.*

Key words: *probiotics, structure of microbial consortia, ribosomal taxonomy, opportunistic pathogens, antagonistic activity*

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ПРОБИОТИЧЕСКИЕ КОНСОРЦИУМЫ: СТРУКТУРА И АНТАГОНИСТИЧЕСКАЯ АКТИВНОСТЬ В ОТНОШЕНИИ УСЛОВНО-ПАТОГЕННЫХ БАКТЕРИЙ И НОРМОБИОТЫ ЧЕЛОВЕКА (НА ПРИМЕРЕ *ESCHERICHIA COLI*) *IN VITRO*

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РЕЗЮМЕ

Актуальность. *Применение пробиотических препаратов на основе консорциумов микроорганизмов не только способствует восстановлению баланса кишечной микробиоты, но и увеличивает терапевтический эффект пробиотиков. Перспективными источниками получения пробиотических консорциумов являются кисломолочные продукты, подвергшиеся естественному сквашиванию при помощи спонтанно сформировавшихся микробных консорциумов.*

Цель работы. *Изучение структуры пяти микробных консорциумов с пробиотическими свойствами из кисломолочных продуктов естественного брожения и оценка их антагонистической активности в отношении условно-патогенных бактерий и представителя нормобиоты человека – Escherichia coli – in vitro.*

Материалы и методы. *Анализ структуры бактериальных консорциумов проводили методами секвенирования. Антагонистическую активность консорциумов оценивали диско-диффузионным методом.*

Результаты. *Установлено, что исследуемые микробные консорциумы представлены бактериями Enterococcus spp. и Streptococcus spp. В консорциумах № 1, № 2 и № 3 доминировали бактерии рода Enterococcus, в то время как в консорциумах № 4 и № 5 – Streptococcus. Показана антагонистическая активность в отношении четырёх изолятов условно-патогенных бактерий: Klebsiella pneumoniae № 493, Enterobacter hormaechei № 372, Staphylococcus aureus № 4 и Pseudomonas aeruginosa № 25 ИМБ, а также одного представителя нормобиоты человека – Escherichia coli № 495. Наибольшая зона задержки роста отмечена у изолята E. coli № 495. У трёх тест-культур (K. pneumoniae № 509, E. coli ATCC 25922 и P. aeruginosa № 3 ИМБ) наблюдался более плотный рост вокруг дисков с пробиотическими консорциумами.*

Заключение. *Результаты исследования показали, что влияние пробиотических консорциумов, отличающихся составом микроорганизмов, может быть нейтральным и бактерицидным. Наличие антагонистической активности у исследуемых микробных консорциумов в отношении полирезистентных изолятов условно-патогенных бактерий – перспектива для создания пробиотиков с антибактериальными свойствами.*

Ключевые слова: *пробиотики, структура микробных консорциумов, рибосомная таксономия, условно-патогенные бактерии, антагонистическая активность*

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OBJECTIVES

An actual way to restore the balance of the microbiota of the gastrointestinal tract is the use of probiotic preparations [1]. Recently, much attention has been paid to consortia of probiotic microorganisms, the use of which makes it possible to achieve the expected positive effects from taking probiotics [2]. In a symbiotic consortium, the biological properties of individual strains are mutually enhanced, which makes it possible to create a single biological system with protective properties against the influence of other microorganisms. Representatives of such genera as *Lactobacillus, Bifidobacterium, Streptococcus, Propionibacterium* and *Enterococcus* are most often of commercial interest among microorganisms with probiotic activity [1].

One of the directions of obtaining multistain preparations is the formation of a natural population of microorganisms. According to research, fermented milk products obtained by natural fermentation can be promising sources of such populations [3–5]. In this case, microorganisms independently form consortia with certain functional properties. Naturally formed populations of microorganisms can have a high degree of stability and synergistic effect, which makes them attractive for use as probiotics. An example of the formation of such populations can be a significant change in the species diversity of starter cultures of microorganisms, which, nevertheless, make it possible to obtain products conforming to GOST [6].

In the screening process of some commercial ferments intended for milk fermentation, despite compliance with the technology and instructions, microbial consortia were obtained, which differ from the declared ones on microscopic examination. At the same time, these consortia retained their probiotic properties, which was an important factor for studying their taxonomic structure and antagonistic activity.

THE AIM OF THE STUDY

To study the structure of five microbial consortia with probiotic properties from naturally fermented milk products and to assess *in vitro* their antagonistic activity against opportunistic bacteria and a representative of the human normobiota – *Escherichia coli*.

METHODS

The objects of research

The objects of the study were five microbial consortia with probiotic properties obtained from fermented dairy products of natural fermentation. 56 bacterial isolates were used as test cultures, of which 16 belong to the intestinal normobiota; 4 are reference strains; 36 are isolates of opportunistic bacteria with multiple antibiotic resistance (ADB), included in the Collection of Human Microbiota of the Irkutsk Region of the Scientific Centre for Family Health and Human Reproduction Problems [7]. Species composition of bacterial test cultures is shown in Table 1.

TABLE 1

SPECIES COMPOSITION OF BACTERIAL TEST CULTURES

Type of microorganism	Number of isolates, abs.
Human normobiota	
Escherichia coli NEA	16
Reference strain	
Escherichia coli ATCC 25922	1
Opportunistic bacteria	
Enterobacter cloacae	4
Enterobacter hormaechei	1
Citrobacter amalonaticus	1
Klebsiella oxytoca	1
Klebsiella pneumoniae	6
Klebsiella pneumoniae	2
Proteus mirabilis	1
Pseudomonas aeruginosa	12
Staphylococcus aureus	3
Escherichia coli WEA	3
Escherichia coli HA	2
Reference strains	
Enterococcus faecalis ATCC 29212	1
Pseudomonas aeruginosa ATCC 10145	1
Staphylococcus aureus ATCC 25923	1

Note. NEA – normal enzymatic activity; WEA – weak enzymatic activity; HA – hemolytic activity.

Study design

1. Study of the structure of five probiotic consortia:

a) sequencing of next generation amplicons (NGS, next generation sequencing);

b) sequencing of the 16S fragment of the ribosomal operon using the Sanger method.

2. Testing of the antagonistic activity of five probiotic consortia against test cultures of opportunistic bacteria and a representative of the human normobiota – *Escherichia coli – in vitro*.

Methods of study

1a. The structure of bacterial consortia was analyzed by high-throughput V3–V4 sequencing of variable fragments of the *16S* rRNA gene. DNA from storage cultures was isolated using a commercial set of Quick-DNA Miniprep Kits (Zymo Research, USA). Amplification of the target fragment was performed on highly conserved bacterial primers NGS318L and NGS806R with adapters (Table 2). The resulting amplicons were purified from primer dimers using AMPure XP (Beckman Coulter, USA) according to the manufacturer's protocol.

Sequencing using Illumina technology was carried out at the Genomic Technologies, Proteomics and Cell Biology of the All-Russian Research Institute of Agricultural Meteorology.

The NGS results were processed and taxonomically annotated using the QIIME2 v. 2022.2 platform and the SILVA 138 nucleotide sequence database.

1b. Identification of the dominant bacteria belonging to the consortium was carried out using ribosomal phylogeny using a site including V3–V8 variable regions of the *16S* rRNA gene. Primers 500F and 1350R were used to amplify this fragment (Table 2). Amplicons obtained by polymerase chain reaction (PCR) were purified in 1% agarose gel and embedded in the pJET1.2 vector according to the manufacturer's protocol (Thermo Fisher Scientific, USA). The annular plasmid was transformed into competent *E. coli* XL-1 cells [8] and direct screening of all grown colonies was performed for the presence of an insert of the required length on plasmid primers pJET1.2-F and pJET1.2-R (Table 2).

The sequence reaction was carried out using reagents Brilliant Dye Cycle Sequencing Kit v. 3.1 (NimaGen, the Netherlands) according to the manufacturer's protocol.

TABLE 2
THE STRUCTURE OF PRIMERS USED IN THE STUDY

Name	Structure (5'-3')	PCR conditions
NGS318F	TCGTCGGCAGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG	Step 1, Cycle = 01 T1 = 95 °C; t = 3 min Step 2, Cycle = 25 T1 = 95 °C; t = 30 s T2 = 55 °C; t = 30 s T3 = 72 °C; t = 30 s Step 3, Cycle = 01 T1 = 72 °C; t = 5 min
NGS806R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCMGGGTATCTAATCCKGTT	
500F	GTGCCAGCAGCCGCGGTAA	Step 1 Cycle = 01 T1 = 95 °C; t = 5 min Step 2, Cycle = 25 T1 = 94 °C; t = 30 s T2 = 60 °C; t = 30 s T3 = 72 °C; t = 1 min Step 3, Cycle = 01 T1 = 72 °C; t = 5 min
1350R	GACGGGCGGTGTGTACAAG	
pJET1.2-F	CGACTCACTATAGGGAGAGCGGC	Step 1, Cycle = 01 T1 = 95 °C; t = 3 min Step 2, Cycle = 25 T1 = 94 °C; t = 30 s T2 = 60 °C; t = 30 s T3 = 72 °C; t = 1 min Step 3, Cycle = 01 T1 = 72 °C; t = 5 min
pJET1.2-R	AAGAACATCGATTTTCCATGGCAG	

Amplicons were sequenced by Sanger on the Nanophor-05 device at the Center for the Development of Progressive Personalized Health Technologies of the Scientific Centre for Family Health and Human Reproduction Problems.

Sequents were adjusted visually in the Bioedit v. 7.2.5 software. Species identification was performed by comparing the nucleotide sequence with the NCBI NR and EMBL-ENA Sequences bases using BLAST and FASTA, respectively.

2. The antagonistic activity of probiotic consortia was determined by the disc diffusion method, according to the standard methodology for determining the sensitivity of microorganisms to antimicrobial preparations [9]. Borosilicate glass filters Glass Microfiber Filters (GF/F) (Whatman plc., UK) with a diameter of 0.5 cm were used as discs, on which suspensions of the tested consortia in a volume of 15 µl were pipetted. The total content of microbial cells in each consortium was 10^{10}–10^{11} CFU/cm^3. Dishes were incubated at 37°C for 24 hours.

The measurement of growth retardation zones (GRZ) of test crops, taking into account the filter diameter, was carried out according to the results of two separate experiments using the ImageJ v. 1.5.3 graphical editor, which allows for image analysis. At GRZ of 6.0 mm and above, the consortium was considered to exhibit antagonism. The data are presented in the form of the arithmetic mean diameters of the growth suppression zones of test cultures (*M*) and the RMS deviation (*m*).

RESULTS AND DISCUSSION

According to the results of high-performance sequencing, *Enterococcus* spp entered the microbial consortia as dominant bacteria. and *Streptococcus* spp. (Table 3).

TABLE 3

STRUCTURE OF MICROBIAL CONSORTIA WITH PROBIOTIC PROPERTIES, %

Characteristics of the taxon	No. of the microbial consortium				
	1	2	3	4	5
Enterococcus	89.8	69.2	51.7	0.2	0.6
Streptococcus	7.2	25.2	36.9	86.6	88.3
Other	2.22	5.32	8.88	10.48	9.95

The proportion of concomitant bacteria ranged from 2.22 to 10.48 %. Consortia No. 1 and No. 2 were dominated by representatives of the genus *Enterococcus*, in consortia No. 4 and No. 5 – *Streptococcus,* while in consortium No. 3 the relative content of representatives of these genera was 51.7 % and 36.9 %, respectively.

Identification of the dominant bacteria based on ribosomal taxonomy showed that two types of enterococci were identified in Consortium No. 1 – *Enterococcus durans* and *Enterococcus thailandicus* (Table 4). Consortium No. 2 turned out to be richer in the diversity of enterococci: in addition to the above-mentioned species, *Enterococcus faecium* was identified in it. In addition, streptococci made up 25.2 % of the structure of the microbial consortium. They have been identified as *Streptococcus salivarius* and *Streptococcus thermophilus*. In consortium No. 3, enterococci are represented by the species *E. durans*, and streptococci by the species *S. salivarius* and *S. thermophilus.* The composition of consortium No. 4 is represented by the *S. thermophilus* monoculture, while *Lactobacillus brevis* was identified in addition to *S. thermophilus* in consortium No. 5.

Phylogenetic analysis showed that for bacteria of the genus *Enterococcus*, the sequences formed either independent branches or together with sequences of typical strains (Fig. 1).

E. durans is represented in the structure of consortia No. 1, No. 2 and No. 3; *E. faecium* is defined in consortium No. 2; *E. thailandicus* – in consortium No. 1.

To assess the ecological and genetic characteristics of enterococci that are part of the studied consortia, a variety of biotopes of homologous strains were analyzed, among which fermented dairy products, saliva, gastrointestinal tract (GIT) of humans and animals, faeces were found (Fig. 2).

Representatives of consortia No. 1, No. 2 and No. 3 showed phylogenetic affinity with isolates isolated from the human intestinal biotope (Fig. 2, highlighted in green), human breast milk and food dairy products (Fig. 2, highlighted in blue) and from the vaginal biotope (Fig. 2, highlighted in red).

The separation of the studied sequences and homologous strains from the NCBI database into fecal, lactic and vaginal biotopes indicates the genetic differences of microorganisms from different ecological groups. Such differences may cause a different degree of manifestation of probiotic properties. The study of the ecological and genetic characteristics of microorganisms with probiotic properties will help to form balanced microbiocenoses for specific human biotopes [10].

Figure 3 shows a phylogenetic tree based on V3–V8 variable fragments of the *16S* rRNA gene for phylotypes assigned to the genus *Streptococcus* and homologous isolates from different biotopes.

There is a difference between representatives of consortia No. 2, No. 4 and No. 5, which have separated into independent branches. The tree shows that the representatives of consortium No. 2 had homology with the *S. salivarius* H4 isolate. Also, all the studied consortia were represented by phylotypes that showed homology with *S. thermophilus isolates.* Homologous isolates were obtained from various dairy products and biotope of the human oral cavity.

TABLE 4

RIBOSOMAL TAXONOMY OF BACTERIA DOMINATED IN MICROBIAL CONSORTIA WITH PROBIOTIC PROPERTIES

Labelling of a clonal sequence	The nearest bacterial homologue	Percentage of homology, %
	Consortium No. 1	
1.10; 1.27	*Enterococcus durans* HBUAS54304	99.4; 100
1.19; 1.28	*Enterococcus durans* IPLA 655	99.5; 99.5
1.20; 1.21; 1.23	*Enterococcus thailandicus* LM4-1	99.4–99.5
1.25	*Enterococcus thailandicus* Marseille-AA00296	99.8
	Consortium No. 2	
2.1	*Enterococcus thailandicus* LM4-1	100
2.2	*Enterococcus thailandicus* Colony540	99.4
2.6	*Enterococcus durans* HBUAS54304	100
2.8	*Streptococcus salivarius* H4	99.4
2.10	*Enterococcus faecium* HBUAS66260	99.6
2.11	*Streptococcus thermophilus* ST106	99.9
2.13	*Enterococcus durans* ABRIINW.N3	99.6
	Consortium No. 3	
3.1	*Streptococcus salivarius* H4	99.1
3.3	*Enterococcus durans* ULAG	98.7
3.4; 3.7; 3.11	*Streptococcus thermophilus* c 21.5	97.8–98.4
3.8	*Enterococcus durans* HBUAS54304	99.4
3.10	*Streptococcus thermophilus* STN57	97.9
	Consortium No. 4	
4.1;	*Streptococcus thermophilus* ASR-1	98.8
4.2; 4.3	*Streptococcus thermophilus* IMAU:80427	99.1; 98.8
4.5	*Streptococcus thermophilus* ChR-I-str19	99.8
4.6; 4.7; 4.8	*Streptococcus thermophilus* BL13-10	99.0–99.8
	Consortium No. 5	
5.1	*Streptococcus thermophilus* ChR-I-str19	99.6
5.3; 5.2; 5.4; 5.5; 5.6; 5.8	*Streptococcus thermophilus* BL13-10	97.9–99.5
5.7	*Streptococcus thermophilus* PT110	84.4

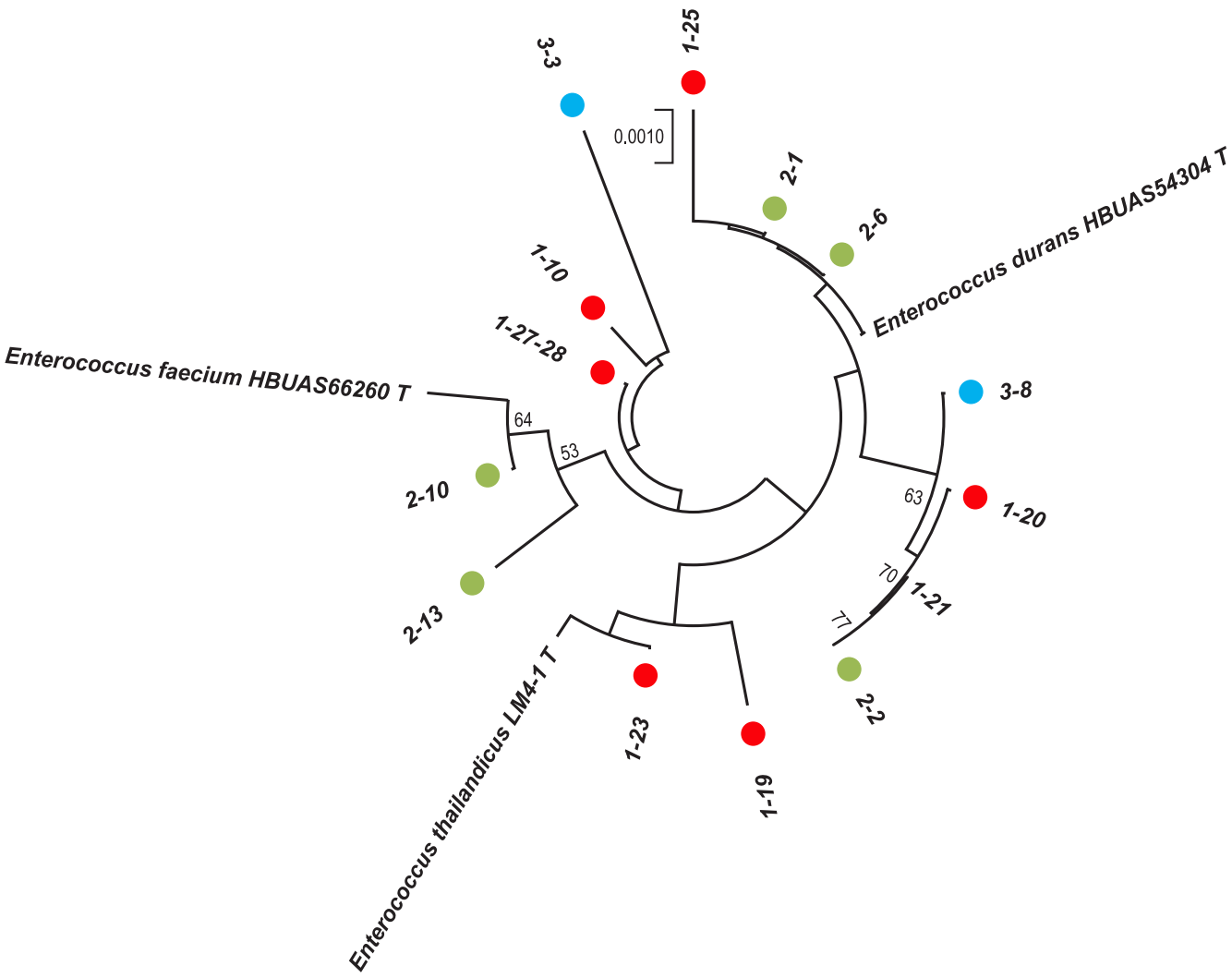


FIG. 1.

Phylogenetic tree based on V3–V8 variable fragments of the 16S rRNA gene for phylotypes assigned to the genus Enterococcus and type strains: the nodes show values of bootstrap support (%) above 50

The presence of antagonistic activity against pathogenic and opportunistic microorganisms (OM) is one of the main characteristics of the strain for classifying it as a probiotic species. The results of the study showed that the effect of the probiotic consortia under study, which differ in the composition of microorganisms, can be neutral and bactericidal: antagonistic activity was noted against five isolates of test cultures (Table 5).

All probiotic consortia suppressed the growth of two isolates: OM – *K. pneumoniae* No. 493 and a representative of the intestinal normobiota – *E. coli* No. 495. The maximum growth suppression zone was noted in *E. coli* isolate No. 495 with consortium No. 3. Consortia No. 1, No. 2, No. 4 and No. 5 exhibited antagonistic activity against *E. hormaechei* No. 372, consortia No. 1, No. 3 and No. 5 – against *S. aureus* No. 4, consortium No. 3 – against *P. aeruginosa* No. 25 IMB.

Three test cultures exhibited more dense growth around filters with probiotic consortia: *K. pneumoniae* isolate No. 509 and the reference strain of *E. coli* ATCC 25922 have discs with all five consortia, isolate *P. aeruginosa* No. 3 IMB – around probiotic consortia No. 1, No. 2, No. 3 and No. 4 (Fig. 4). This fact requires further detailed study using techniques designed to evaluate the growth-stimulating properties of bacteria.

Recently, quite a lot of papers have been published on the use of probiotics based on *Streptococcus* spp. and *Enterococcus* spp. and their beneficial properties for the human body. Probiotic strains of enterococci and streptococci are widely used to correct human intestinal dysbiosis, as well as in chronic gastrointestinal diseases [11, 12]. *S. thermophilus* strain belongs to the group of lactic acid bacteria that ferment sugars to lactic acid, exerting an acidifying effect and providing a bactericidal effect against many pathogenic microorganisms [13]. Enterococcal cultures have long been used for cooking meat, milk and vegetables. The low content of enterococci in meat and dairy products does not allow pathogenic staphylococci and *E. coli* to multiply [14]. It is known that the antibacterial activity of enterococci is associated with their ability to synthesize specific proteins – enterocins A, B, L50A/B, P, Q and Xa/β. The presence of such proteins determines the antagonistic activity of enterococci against infectious

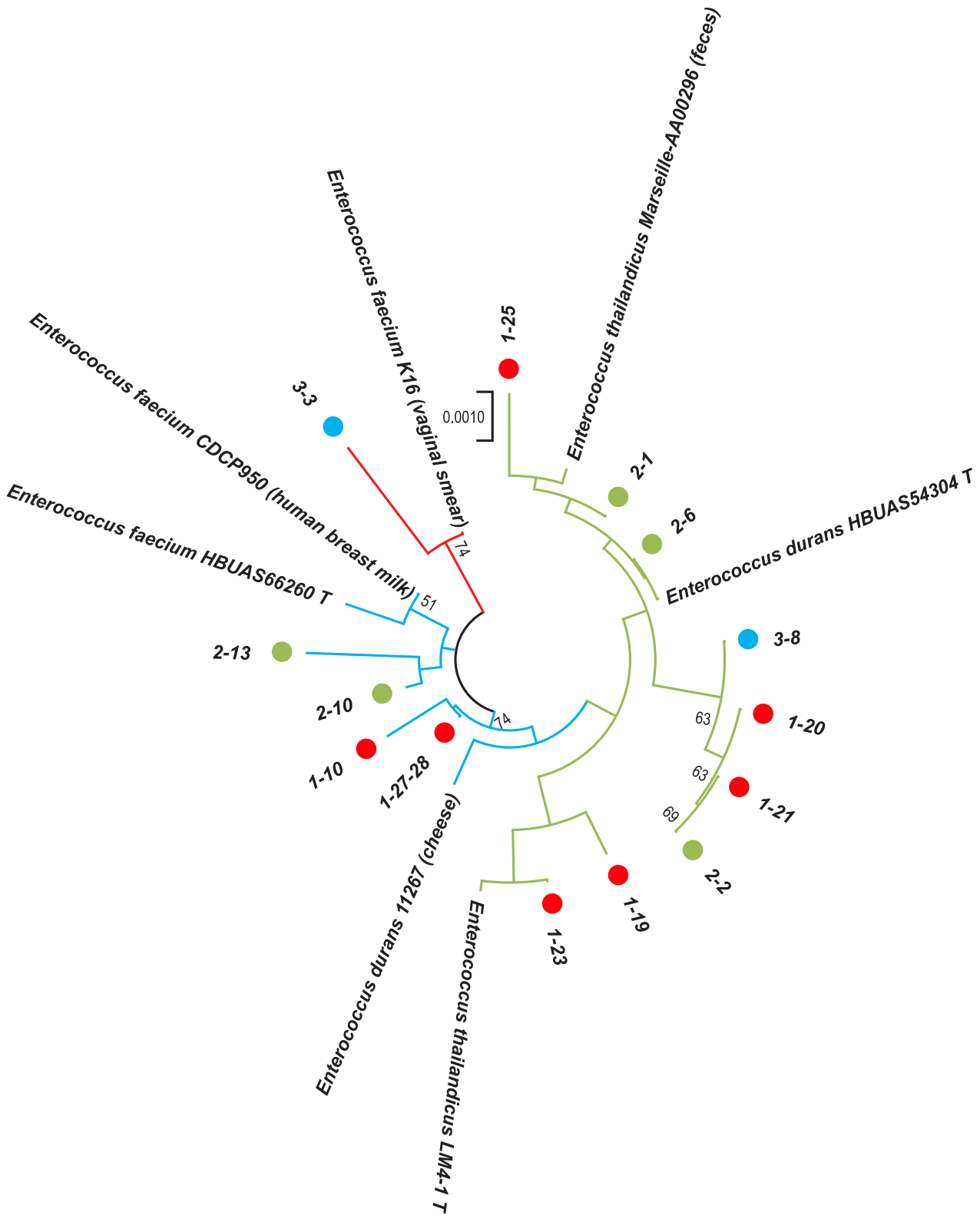


FIG. 2.

Phylogenetic tree based on V3–V8 variable fragments of the 16S rRNA gene for phylotypes assigned to the genus Enterococcus, type strains and homologous strains from various biotopes: the nodes show values of bootstrap support (%) above 50

agents such as *S. aureus*, *E. coli*, as well as *Klebsiella* spp., *Enterobacter* spp., *Streptococcus* spp., *Listeria* spp. and *Clostridium* spp. [15, 16]. The data obtained by us on the antagonistic activity of probiotic consortia based on *Streptococcus* spp. and *Enterococcus* spp. correspond to the data found in the literature. However, the tested isolates have multiple antibiotic resistance, which means they are more adapted to the effects of negative environmental factors. Probably, this can explain the selective sensitivity of resistant isolates of one type of OM to the action of probiotic consortia.

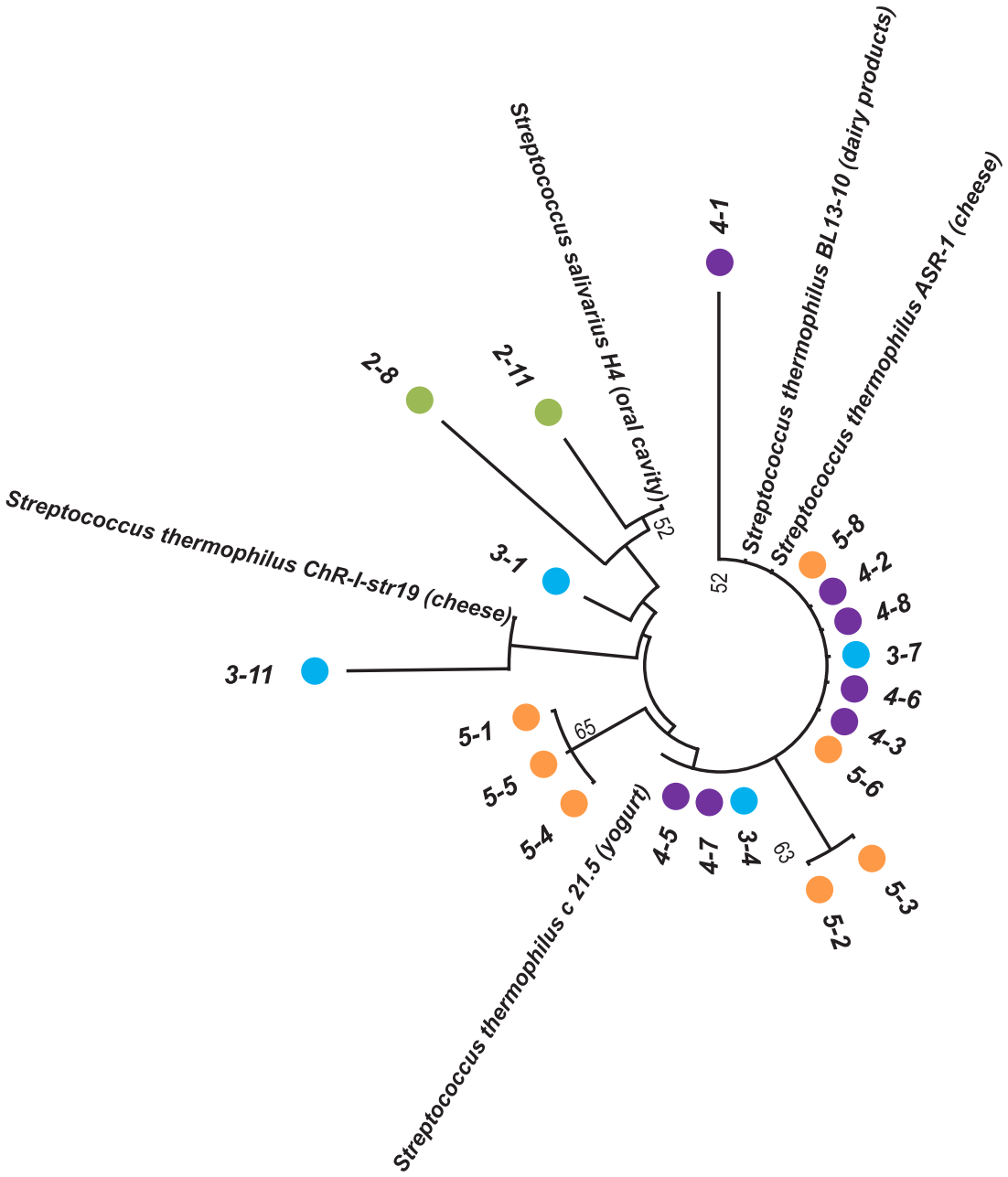


FIG. 3.
Phylogenetic tree based on V3–V8 variable fragments of the 16S rRNA gene for phylotypes assigned to the genus Streptococcus and homologous strains: the nodes show values of bootstrap support (%) above 50

CONCLUSION

In this study, the taxonomic structure and antagonistic activity of five microbial consortia with potential probiotic properties were investigated. Using molecular genetic methods, it was found that the studied microbial consortia are represented by *Enterococcus* spp. and *Streptococcus* spp. bacteria. n consortiums No. 1, No. 2, and No. 3, *Enterococcus* bacteria dominated, while in consortiums No. 4 and No. 5, *Streptococcus* dominated. Representatives of the genus *Enterococcus* were identified as *E. durans*, *E. thailandicus*, *E. faecium*; the genus *Streptococcus* was represented by the species *S. salivarius* and *S. thermophilus*.

It was shown that the effect of probiotic consortia, differing in the composition of microorganisms, can be neutral and bactericidal. The probiotic consortia studied suppressed the growth of four isolates of opportunistic bacteria, such as *Klebsiella pneumoniae* No. 493, *Enterobacter hormaechei* No. 372, *Staphylococcus aureus* No. 4 and *Pseudomonas aeruginosa* No. 25, as well as one representative of the human normobiota, *Escherichia coli* No. 495. The largest growth retardation zone was observed in *E. coli* isolate No. 495 in the presence of consortium No. 3.

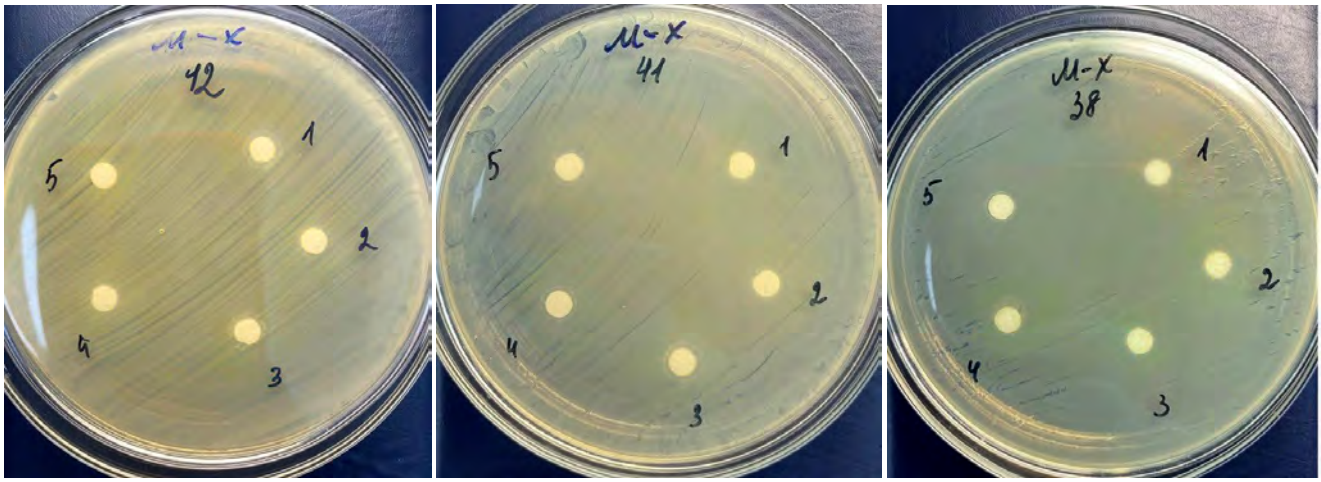
The presence of antagonistic activity in the studied microbial consortia in relation to polyresistant isolates of opportunistic bacteria may be a prospect for the crea-

TABLE 5

ANTAGONISTIC ACTIVITY OF PROBIOTIC CONSORTIA IN RELATION TO OPPORTUNISTIC BACTERIA AND HUMAN NORMOBIOTA

No.	Type of microorganism (isolate number) / characteristic [a]	Growth retardation zones, mm (M ± m)				
		No. 1	No. 2	No. 3	No. 4	No. 5
1	*Proteus mirabilis* (No. 371) / OM, ADB	0	5.5 ± 0.2	5.5 ± 0.2	0	0
2	*Enterobacter hormaechei* (No. 372) / OM, ADB	6.7 ± 0.3[b]	6.2 ± 0.2[b]	5.7 ± 0.2	6.3 ± 0.3[b]	7.0 ± 0.2[b]
3	*Klebsiella pneumoniae* (No. G) / OM, ADB	5.4 ± 0.1	5.7 ± 0.2	5.6 ± 0.2	5.4 ± 0.2	5.5 ± 0.2
4	*Enterobacter cloacae* (No. 394) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	0	5.2 ± 0.1
5	*Klebsiella oxytoca* (No. 439) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.2	5.2 ± 0.1	5.2 ± 0.1
6	*Klebsiella pneumoniae* (No. 493) / OM, ADB	6.8 ± 0.[3]b	6.4 ± 0.3[b]	6.8 ± 0.3[b]	6.9 ± 0.3[b]	6.6 ± 0.3[b]
7	*Klebsiella variicola* (No. 672) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
8	*Staphylococcus aureus* (No. 672), / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
9	*Staphylococcus aureus* (No. 4) / OM, ADB	6.2 ± 0.3[b]	5.9 ± 0.1	6.3 ± 0.3[b]	5.2 ± 0.1	6.5 ± 0.3[b]
10	*Klebsiella pneumoniae* (No. 41HE) / OM, ADB	5.4 ± 0.1	5.6 ± 0.3	5.4 ± 0.2	5.6 ± 0.2	5.3 ± 0.1
11	*Staphylococcus aureus* (No. 846) / OM, ADB	0	5.4 ± 0.3	5.2 ± 0.1	0	5.2 ± 0.1
12	*Klebsiella pneumoniae* (No. 381) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.4 ± 0.2	5.2 ± 0.1
13	*Pseudomonas aeruginosa* (No. 25 IMB) / OM, ADB	0	5.8 ± 0.1	6.3 ± 0.3[b]	0	5.9 ± 0.1
14	*Pseudomonas aeruginosa* (No. 54 IMB) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.2	5.7 ± 0.2	5.2 ± 0.1
15	*Pseudomonas aeruginosa* (No. 82 IMB) / OM, ADB	5.2 ± 0.1	0	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
16	*Pseudomonas aeruginosa* (No. 3 IMB) / OM, ADB	+	+	+	+	5.2 ± 0.1
17	*Pseudomonas aeruginosa* (No. 5 IMB) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.3 ± 0.2
18	*Enterobacter cloacae* (No. 25) / OM, ADB	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.5 ± 0.2	5.2 ± 0.1
19	*Klebsiella pneumoniae* (No. 509) / OM, ADB	+	+	+	+	+
20	*Escherichia coli* (No. 473) / ADB	5.2 ± 0.2	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.2	5.2 ± 0.1
21	*Escherichia coli* (No. 495) / ADB	7.2 ± 0.3[b]	7.9 ± 0.3[b]	8.2 ± 0.3[b]	7.4 ± 0.3[b]	7.3 ± 0.3[b]
22	*Escherichia coli* (No. 6G) / normobiota	5.2 ± 0.1	5.2 ± 0.2	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
23	*Escherichia coli* (No. 133HE) / normobiota	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.2	0	5.2 ± 0.1
24	*Escherichia coli* (ATCC 25922) / reference strain	+	+	+	+	+

Note. [a] – characteristic of the isolate; OM – opportunistic microorganism; ADB – presence of multiple antibiotic resistance; 0 – no effect; + – denser growth of the test culture around the filter; [b] – the consortium exhibited antagonism.



a ***b*** ***c***

FIG. 4.

Growth of test cultures around filters with probiotic consortiums: ***a*** *– Klebsiella pneumoniae No. 509;* ***b*** *– Escherichia coli ATCC 25922;* ***c*** *– Pseudomonas aeruginosa No. 3 IMB;* 1 *– consortium No. 1;* 2 *– consortium No. 2;* 3 *– consortium No. 3;* 4 *– consortium No. 4;* 5 *– consortium No. 5*

tion of probiotic preparations with antibacterial properties in conditions of widespread drug resistance of microorganisms.

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Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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