



Specific properties of probiotic strains: relevance and benefits for the host

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Abstract

Background Probiotics have tremendous potential to develop healthy diets, treatment, and prevention. Investigation of in vitro cultural properties of health-promoting microorganisms like lactic acid bacteria (LAB) and bifidobacteria is crucial to select probiotic strains for treatments based on gut microbiota modulation to justify individualized and personalized approach for nutrition and prevention of variety of diseases. The *aim* was to study the biological properties of LAB and bifidobacteria probiotic strains, namely adhesive properties; resistance to antibiotics; and biological fluids (gastric juice, bile, pancreatic enzymes), and to overview the literature in the field.

Materials and methods We studied six LAB strains (*Lactobacillus acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. rhamnosus* LB-3 VK6, *L. delbrueckii* LE VK8, *L. plantarum* LM VK7), and two bifidobacteria strains (*Bifidobacterium animalis* VKL, *B. animalis* VKB). We characterized tinctorial, culturally morphological, physiological, and biochemical properties of probiotic strains of LAB and bifidobacteria by commonly used research methods. Determination of the resistance to antibiotics was carried out using disc-diffusion method. The effects of gastric juice, bile, and pancreatin on the viability of LAB and bifidobacteria were evaluated. Adhesive properties of LAB and bifidobacteria to epithelial cells were assessed calculating three indicators: average adhesion rate (AAR), participation rate of epithelial cells (PRE), and adhesiveness index of microorganisms (AIM). Electron microscopy of LAB and bifidobacteria cells was conducted.

Results The studied strains of LAB and bifidobacteria did not form spores, were positively stained by Gram, grow on medium in a wide range of pH (1.0–9.0, optimum pH 5.5–6.5), were sensitive to a wide range of antibiotics; and showed different resistance to gastric juice, bile, and pancreatic enzymes. The most resistant to antibiotics were *L. rhamnosus* LB-3 VK6 and *L. delbrueckii* LE VK8 strains. The most susceptible to gastric juice was *L. plantarum* LM VK7, which stopped its growth at 8% of gastric juice; *L. acidophilus* IMV B-7279, *B. animalis* VKL, and *B. animalis* VKB strains were resistant even in the 100% concentration. Strains *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *B. animalis* VKL, *B. animalis* VKB, *L. rhamnosus* LB-3 VK6, *L. delbrueckii* LE VK8, and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 were resistant to pancreatic enzymes. Adhesive properties of the strains according to AIM index were high in *L. casei* IMV B-7280, *B. animalis* VKL, and *B. animalis* VKB; were moderate in *L. delbrueckii* subsp. *bulgaricus* IMV B-7281; and were low in *L. acidophilus* IMV B-7279, *L. rhamnosus* LB-3 VK6, *L. delbrueckii* LE VK8, and *L. plantarum* LM VK7.

Conclusion We recognized strain-dependent properties of studied LAB and bifidobacteria probiotic strains (adhesive ability, resistance to antibiotics, and gut biological fluids) and discussed potential for most effective individualized treatment for gut and distant sites microbiome modulation.

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Overview

Relevance of in vitro research to support strains stratification for effective personalized probiotic interventions

Intestinal microbial population largely represented by Bacteroidetes and Firmicutes, has been proven to impact on human health and maintaining homeostasis [1].

The definition of a probiotic as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” was determined by Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 2001 [2] and confirmed in 2014 by the International Scientific Association for Probiotics and Prebiotics (ISAPP) experts [3] and later remain unchanged being agreed in the broad expert communities. Probiotics have tremendous potential to develop healthy diets and integrated approach for immunity-related disease treatment and prevention [3–12] and are effective actors in the gut and in distant sites [12] with strong potential for applications in personalized medicine and nutrition [13, 14]. Modification of the gut microbiota in chronic diseases and metabolic syndrome [14, 15] is among the leading tasks of microbiome research and needs for clinical use of probiotics [16].

However, *evidence-supported knowledge* on probiotics contribution to disease pathophysiology and applicability to clinical care is *not yet sufficient* [17], excluding very few aspects. Thus, in cases of antibiotic- and *Clostridium difficile*-associated diarrhea and respiratory tract infections, the effects of probiotics are considered “evidence-based” [18].

Studies conducted in vitro and in vivo, including probiotics mechanism of function, gut microbiota composition ecology, and metabolomic researches in regards to screening strains for clinical application are needed to implement personalized probiotic treatment to the clinical care and set relevant designs of clinical trials of particular strains [8, 19, 20]. Since clinical studies are very complicated to design and conduct, in vitro [21] and animal models [22] research still can provide a high quality data in this matter.

Recently *Koch's original postulates* have been adapted to identify microorganisms that contribute to human health and formulated necessary requirements for a microorganism to be considered a probiotic as follows [23]:

1. The strain of the commensal microorganism is associated with the health of the host, which is regularly manifested in healthy hosts, but less common in patients with disease.
2. The strain of the commensal microorganism can be identified as pure culture and cultivated in the laboratory.
3. A strain of a commensal microorganism improves or alleviate the disease when introduced into a new host organism.
4. The strain of the commensal microorganism can be detected after its introduction into a host to which health was restored.

One of the principle mechanisms of probiotics' role in the pathogenesis of a number of diseases is the ability of bacterial strains to contribute to immune response, prevent colonization of pathogen microorganisms, metabolize nutrients and process toxic metabolites, and regulate energy balance [1, 2]. Probiotics demonstrate antagonistic relations with pathogenic and opportunistic bacteria due to the synthesis of a number of organic acids, which leads to pH lowering, hydrogen peroxide, lysozyme, and bacteriocins—polypeptide antibiotic-like substances, which differ in the strength and spectrum of antibiotic action [2, 3, 24].

In addition, probiotic strains are able to synthesize digestive enzymes (amylase, lipase, proteases, pectinases, and endoglucanases) and vitamins A, B, PP, E, C, K, and others [25, 26] and produce metabolites such as short-chain fatty acids and histamine [27]. Due to the effects of these substances, probiotic strains are able to restore microbiota; participate in the metabolism of glucose, cholesterol, bilirubin, choline, bile, and fatty acids; as well as indirectly influence on the metabolism of iron and calcium and to demonstrate immunomodulatory and antitoxic properties [28]. Also, due to the adhesion to the mucosa and epithelium, inhibiting pathogen adhesion and/or growth [28] probiotic strains can prevent lesions of tissues by pathogenic and opportunistic microorganisms.

On the other hand, a broad use of antibiotics alters host phenotypes and gut microbiota and glucose metabolism [29]. In recent decades, uncontrolled antibiotic therapy led to the formation of associations of microorganisms with increased virulence, in particular the so-called hospital strains. Gut microbiota is known to be a potential reservoir of *antimicrobial resistance (AMR) genes*, demonstrating the ability for the horizontal transfer to potential pathogenic bacteria within this ecosystem [30]. Microorganisms AMR are extensively studied over the past decades as so-called “resistome.” Antibiotic susceptibility of probiotic strain can be an important specific indicator; antimicrobial resistance was studied for LAB and bifidobacterium strains [31].

Thus, each individual strain may demonstrate multiple mechanisms of action and have specific phenotype/genotype accordingly; these relevant markers has not been formulated yet for traditional probiotic strains [10] and a comprehensive understanding of these mechanisms full discovered.

Thus, insufficient viability and survival of probiotic bacteria remain a problem in commercial products of fermented food [32]. Therefore, cultural properties of strains are crucial for stratification before inclusion of probiotic bacteria as therapeutic fermented products by selecting best functional probiotic strains and improvement methods of increasing survival, using appropriate prebiotics and the development optimal combination of probiotics and prebiotics [4] (synbiotics) an increasing viable strains delivery of bacteria is essential task [32].

Despite extensive agenda of genomic research in microbiome, *metagenomic studies* have been recently revived in particular as used to the human gut [33–35]. Browne et al. suggested that the gut microbiota is largely *culturable* and the majority of strains can be cultured using a single bacteriological medium [36]. Such “microbial culturomics” approach [33] opens new insights for phenotypic analysis of the human gut microbiota and probiotic strains.

The tests conducted *in vitro* have widely demonstrated the strain-dependent immunomodulation potential of bifidobacteria [37, 38]. *In vitro* models have important limitations but they enable the preliminary screening of the effects that bacterial cells or fractions might have on different components of the immune response [39, 40], e.g., *in vitro* models using immune cells as macrophage-like cell lines for bifidobacteria and lactic acid bacteria (LAB) [39] and cells isolated from the gut-associated lymphoid tissues (GALT) [40]. *In vitro* properties like the cell wall parameters [39, 40] play an essential role in many aspects of modulating beneficial immune response and EPS-producing phenotype can depend on a single gene (e.g., *Balat_1410* for *Bifidobacterium animalis* subsp. *lactis*) [41]. Such established crosslinks between phenotype-genotype can warrant to stratify strains on their modulatory activity on innate immunity to justify individualized and personalized approach for nutrition and prevention.

Lactobacillus and bifidobacterium strains are the most commonly used probiotics [42]; although *their* cultural properties have not been widely used to examine the immunomodulation potential to select probiotic strains for individualized treatments based on gut microbiota modulation in close regards to patient’s phenotype analysis.

The *aim* was to investigate the biological properties of *Lactobacillus acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. rhamnosus* LB-3 VK6, *L. plantarum* LM VK7, *L. delbrueckii* LE VK8, *B. animalis* VKL, and *B. animalis* VKB strains, namely adhesive properties, resistance to

antibiotics and biological fluids (gastric juice, bile, pancreatic enzymes); to overview the literature in the field and discuss the potential for individualized use in adherence with patient’s needs according to host phenotype to obtain the best effect possible.

Materials and methods

Bacterial strain, media, and growth conditions

The objects of the study were as follows:

Six LAB strains (*L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. rhamnosus* LB-3 VK6, *L. delbrueckii* LE VK8, *L. plantarum* LM VK7)

and two strains of the *Bifidobacterium* genus (*B. animalis* VKL, *B. animalis* VKB).

Strains used in our study are deposited in the Ukrainian collection of microorganisms (Zabolotny Institute of Microbiology and Virology of NAS of Ukraine, Kyiv, Ukraine).

No human participant were included and no animals used in this study.

Studies were performed using microorganisms, freeze-dried in a Cuddon Freeze Dryer FD1500 (New Zealand). Before each experiment, the viability of the probiotic strains was tested by monitoring their growth on the Man-Rogosa-Sharpe (MRS) agar or bifidum agar (BA) in aerobic and anaerobic conditions, respectively, at 37 °C for 24–48 h. The same cultural media without agar adding were used to determine the growth curve of bacterial cultures.

Determination of the resistance to antibiotics of different groups was carried out on MRS agar (MRSA) and BA mediums by disc-diffusion method [19, 22]. Standardized discs with antibiotics (Obolensk, Russia) were used. The degree of bacteria sensitivity to antibiotics was determined by the size of growth inhibition zone: less than 10 mm was evaluated as resistant (*R*); 10–20 mm as medium resistant (*M*); and over 20 mm as sensitive (*S*) [31].

The effects of gastric juice, bile, and pancreatin on the viability of LAB and bifidobacteria were determined. Daily bacterial strains were grown in liquid media at 37 °C with gastric juice in a concentration of 1, 2, 5, 8, 10, 20, 30, 50, 75, and 100% (for 2.5 h) or in bile in a concentration of 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5% (for 5 h) or pancreatin at a concentration of 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5% (for 15 h). After this, LAB and bifidobacteria were sown to MRSA and BA, respectively. After 24–48 h cultivation at 37 °C with high content of carbon dioxide in the air (5%), the number of colony-forming units (CFU) was counted. Complex effect of

gastric juice, bile, and pancreatin on the activity of LAB and bifidobacteria was evaluated. For this purpose, the strains were grown in MRS and bifidum media, respectively, with the gradual addition of 2% of gastric juice (for 2.5 h), 1% of bile (for 5 h), and 1% of pancreatin (for 15 h) [43, 44]. Determination of adhesive properties of probiotic strains was performed by standard method as suggested in [45].

Adhesive properties of LAB and bifidobacteria were assessed by three indicators: the average adhesion rate (AAR; the average number of microorganisms that have attached to the surface of one epitheliocyte in case of counting no less than 100 epithelial cells, given no more than five in a single field of view); the participation rate of epithelial cells (PRE; percent of epithelial cells, having on its surface-adhered microorganisms), the adhesiveness index of microorganisms (AIM; the average number of microorganisms on one epitheliocyte that participate in the adhesive process). AIM is calculated by the formula [45]:

$$\frac{\text{AAR}}{\text{PRE}} \cdot 100\%$$

It was considered that microorganisms had no adhesive activity at AIM index ≤ 1.75 ; had low adhesive activity at AIM 1.75–2.5, average adhesive activity at AIM 2.51–4.0, and high adhesive activity at AIM > 4.0 [29].

For electron microscopy, daily culture of lactobacilli and bifidobacteria cells were twice washed after cultivation on MRSA or BA media by centrifugation at 2000 rpm and resuspended in a physiological solution. The concentration of microorganisms was adjusted to 1×10^6 cells/ml. The electron microscopy of the samples was carried out by the generally accepted method [46] using the JEM-1400 electron microscope (Zabolotny Institute of Microbiology and Virology, NAS of Ukraine) at 80 kV.

Statistical analysis

Statistical analysis was performed by using one-way analysis of variance (ANOVA) using Epi Info software (USA, version 8.0) and Microsoft Office Excel. Numeric data were presented as mean arithmetic values and their standard deviations ($M \pm m$). For single comparisons, values of P were determined using Student's t test. Differences between groups were defined significant at $P < 0.05$.

Results

We established tinctorial, culturally morphological, physiological, and biochemical properties of probiotic strains of LAB and bifidobacteria by commonly used research methods.

L. acidophilus IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. rhamnosus* LB-3 VK6, *L. plantarum* LM VK7, *L. delbrueckii* LE VK8, *B. animalis* VKL, and *B. animalis* VKB strains were previously obtained from fermented biological material of the intestines of clinically healthy adults. All studied strains were motionless. LAB had a rod-shaped form. Bifidobacteria were characterized by cell polymorphism when the cell's shape depended on the stage of development of the culture and varied from the rod-shaped to the pin-shaped, spindle-shaped, amorphous, and Y- or X-shaped cells (Fig. 1).

The studied strains of LAB and bifidobacteria did not form spores, were positively stained by Gram and grow on medium in a wide range of pH (1.0–9.0, optimum pH 5.5–6.5).

For further investigation of biological properties of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. rhamnosus* LB-3 VK6, *L. plantarum* LM VK7, *L. delbrueckii* LE VK8, *B. animalis* VKL, and *B. animalis* VKB, we studied the dynamics of their growth in the liquid media. Based on the obtained results, we constructed the growth curves of each culture and determined the beginning of the stationary phase of growth in each particular case. The stationary growth phase of *L. delbrueckii* subsp. *bulgaricus* IMV V-7281, *L. delbrueckii* LE VK8, and *L. plantarum* LM VK7 after inoculation in MRS medium and incubation at 37 °C occurred only at 11–12 h. It was found that during growth in the MRS medium, the stationary growth phase in *L. acidophilus* IMV B-7279, *B. animalis* VKL, and *B. animalis* VKB occurred approximately 8 h after the inoculation of these strains. For *L. casei* IMV B-7280 and *L. rhamnosus* LB-3 VK6 strains, the stationary growth phase began only at 10 h after strains inoculation.

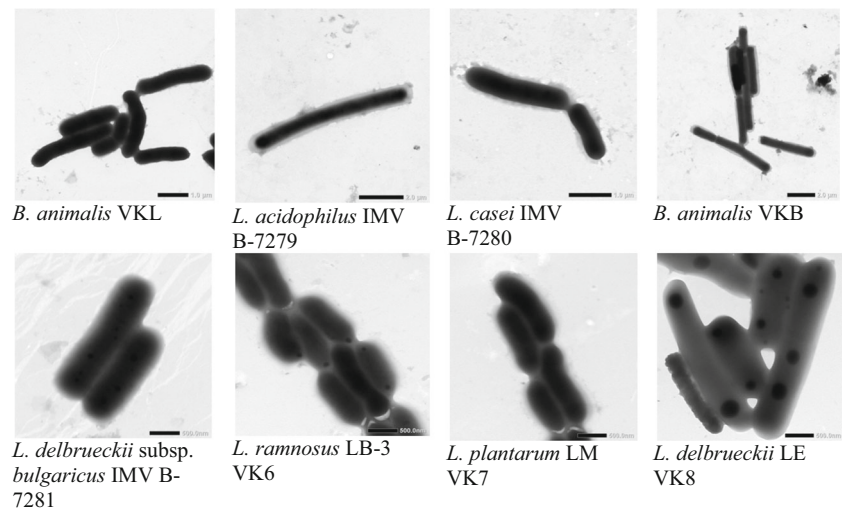
Thus, it has been determined that the beginning of the stationary phase of growth in all strains studied was 7–12 h from the beginning of the growth of culture and was strain-dependent. In the studied bifidobacteria, it occurred a little earlier than in LAB strains.

Resistance to antibiotics

As a result of our study, it was determined the resistance of probiotic strains of LAB and bifidobacteria to antibiotics of different groups with the aim of developing recommendations about the advisability of their further use during antibiotic treatment. The obtained data are provided in Table. 1.

We have established that the following strains were sensitive for most of the investigated antibiotics which effects associated with the blocking of peptidoglycan synthesis: *L. acidophilus* IMV B-7279 (except oxacillin); *L. casei* IMV B-7280; or *B. animalis* VKB (except penicillin, oxacillin, meropenem, and imipenem). *L. rhamnosus* LB-VK6 3 strain was resistant to most inhibitors of peptidoglycan synthesis, while *L. delbrueckii* LE VK8 strain was mostly moderately

Fig. 1 Subcellular imaging (electronic microscopy) of the strains *B. animalis* VKL, *B. animalis* VKB, *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. rhamnosus* LB-3 VK6 (IMV B-7038), *L. plantarum* LM VK7, and *L. delbrueckii* LE VK8. The scale is shown in the images



sensitive to these antibiotics. At the same time, all studied strains were resistant to vancomycin, teicoplanin, and oxacillin. Studied strains had different sensitivities for antibiotics of cephalosporin group, in which mechanism of action is also inhibition of cell wall synthesis. Highly sensitive to the effects of most cephalosporins was *L. acidophilus* IMV B-7279, to a lesser extent—*L. delbrueckii* subsp. *bulgaricus* IMV B-7281. Other strains were resistant or moderately resistant.

Highly sensitive to the effect of antibiotics that are inhibitors of protein synthesis were strains *L. plantarum* LM VK7, *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280 and *B. animalis* VKB (except clarithromycin, roxithromycin, chlortetracycline). Highly sensitive to the action of aminoglycosides, which mechanism of action is also inhibition of protein synthesis, was *L. acidophilus* IMV B-7279 strain only. Other strains were mostly moderately resistant to aminoglycosides.

Nitrofurans had different effects on the studied probiotic strains. Thus, *L. acidophilus* IMV B-7279 strain was highly sensitive, *B. animalis* VKB was moderately resistant, and *L. casei* IMV B-7280 and *L. rhamnosus* LB-3 VK6 strains were resistant. *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 strain was sensitive to furadonin. *L. delbrueckii* LE VK8 and *L. plantarum* LM VK7 strains were resistant to the action of fusidin and furazolidone, but moderately resistant to furadonin.

Inhibitors of nucleic acid synthesis also had different degrees of effect on the studied strains. All strains were resistant to nalidixic acid. *L. delbrueckii* LE VK8, *L. plantarum* LM VK7, and *L. casei* IMV B-7280 strains were mostly moderately resistant to the action of inhibitors of nucleic acids synthesis, whereas *L. rhamnosus* LB-3 VK6 strain was resistant. *B. animalis* VKB was highly sensitive to sparfloxacin, *L. acidophilus* IMV B-7279 to sparfloxacin and ciprofloxacin, and *B. animalis* VKL to sparfloxacin and levofloxacin.

Thus, the investigated strains of LAB and bifidobacteria were sensitive to a wide range of antibiotics. The most resistant to the studied drugs were *L. rhamnosus* LB-3 VK6 and *L. delbrueckii* LE VK8 strains. Therefore, the sensitivity of the studied strains to antibiotics was strain-dependent by nature and did not depend on the genus and species of microorganism.

Resistance to biological agents

The next step was to study the resistance of lactobacilli and bifidobacteria to the action of gastric juice, bile, pancreatin, as well as their complex effect.

In accordance with Fig. 2, all tested strains showed different resistance to gastric juice. The most susceptible was *L. plantarum* LM VK7, which stopped its growth at 8% of gastric juice. The next gastric juice-sensitive strain was *L. delbrueckii* LE VK8, which growth was completely inhibited by gastric juice at a concentration of 10%, with significant growth inhibition already observed at 1% concentration. Complete inhibition of *L. delbrueckii* subsp. *bulgaricus* IMV-7281 growth was observed at a 10% gastric juice concentration in a nutrient medium.

The average resistance to gastric juice had *L. rhamnosus* LB-3 VK6 and *L. casei* IMV B-7280 strains. Growth of *L. rhamnosus* strain LB-3 VK6 was completely suppressed at 50% gastric juice concentration. Growth of the *L. casei* IMV B-7280 strain was not observed after its cultivation in a 100% gastric juice. Strains *L. acidophilus* IMV B-7279, *B. animalis* VKL, and *B. animalis* VKB were resistant to gastric juice even in the 100% concentration.

Probiotic strains of LAB and bifidobacteria have shown different resistance to bile (Fig. 3). The most sensitive was strain *L. delbrueckii* LE VK8, its complete inhibition was observed at a concentration of 8% bile.

Table 1 The resistance of investigated strains of LAB and bifidobacteria to antibiotics

Mechanism of action	Group of antibiotic		Strain of bacteria										
	Name	Generation	<i>B. animalis</i> VKL	<i>B. animalis</i> VKB	<i>L. acidophilus</i> IMV B-7279	<i>L. casei</i> IMV B-7280	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281	<i>L. rhamnosus</i> LB-3 VK6	<i>L. plantarum</i> LM VK7	<i>L. delbrueckii</i> LE VK8			
Inhibition of cell wall synthesis	Penicillins	I II	Penicillin	M	M	S	S	M	R	S	M		
			Oxacillin	R	R	R	R	R	R	M	R		
			Ampicillin	S	S	S	S	M	R	M	M		
			Carbencillin	S	S	S	S	S	R	S	S		
			Azlocillin	S	S	S	S	S	R	S	M		
			Piperacillin	M	S	S	S	S	R	M	M		
			Amoxicillin	S	S	S	S	S	R	S	M		
			Amoxicillin/clavulanic acid	S	S	S	S	S	R	S	M		
			Ticarcillin	M	S	S	S	S	R	S	M		
			Ticarcillin/clavulanic acid	M	S	S	S	S	M	S	S		
			Cephalosporins	I II III IV	Cefazolin	R	M	M	M	M	R	M	R
					Cefalexin	M	M	M	M	R	M	M	R
					Cefuroxime	M	R	M	M	R	R	M	M
Cefaclor	M	S			M	M	M	M	R	M			
Cefotaxime	R	S			S	M	S	R	S	R			
Ceftriaxone	M	M			S	M	S	R	M	M			
Cefoperazone	M	M			M	M	S	R	M	M			
Ceftazidime	R	M			S	R	R	R	R	R			
Cefamandole	M	R			S	S	S	S	R	M			
Cefibuten	R	R			R	M	R	R	R	R			
Cefepime	R	M			M	M	R	R	R	R			
Glycopeptides	Vancomycin Teicoplanin	R			R	R	R	R	R	R	M		
		R	R	R	R	R	R	R	M				
Carbapenems	Imipenem Meropenem	M	S	S	M	M	M	M	M				
		S	S	S	M	M	M	M	M				
Inhibition of protein synthesis	Aminoglycosides	S	S	S	M	M	M	M	M				
		S	S	S	M	M	M	M	M				

Table 1 (continued)

Mechanism of action		Group of antibiotic		Strain of bacteria							
Name		<i>B. animalis</i> VKL	<i>B. animalis</i> VKB	<i>L. acidophilus</i> IMV B-7279	<i>L. casei</i> IMV B-7280	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281	<i>L. rhamnosus</i> LB-3 VK6	<i>L. plantarum</i> LM VK7	<i>L. delbrueckii</i> LE VK8		
Antibiotic resistance/diameter of growth inhibition zone											
I	Streptomycin	M	M	S	S	M	R	M	M		
	Neomycin	S	M	S	M	R	M	M	M		
	Kanamycin	R	R	S	M	R	R	R	M		
	Garamycin	S	S	S	M	M	M	S	M		
	Tobramycin	R	S	S	M	M	M	S	M		
	Sisomicin	M	M	S	S	M	M	S	S		
	Amikacin	M	M	S	M	R	M	R	R		
	Netilmicin	M	M	S	M	M	R	M	M		
	Macrolides										
	I	Oleandomycin	S	S	S	S	M	R	S	M	
II	Erythromycin	M	S	S	S	M	R	S	S		
	Azithromycin	R	S	S	S	M	R	S	S		
	Clarithromycin	S	R	S	S	S	R	S	S		
	Roxithromycin	M	M	S	S	S	R	S	M		
Lincosamides											
I	Lincomycin	M	S	S	S	M	M	S	S		
II	Clindamycin	M	S	S	S	S	R	S	M		
Tetracyclines											
I	Tetracycline	M	S	S	M	M	S	S	M		
I	Chlortetracyclin	M	R	S	S	S	S	S	S		
II	Doxycycline	M	S	S	M	S	R	S	M		
Nitrofurans											
Violations of the synthesis of respiratory enzymes and biosynthesis of cell membrane proteins											
I	Nitrofurantoin	R	M	S	R	S	R	M	M		
	Furazolidone	R	M	S	R	M	R	R	R		
	Fusidin	M	M	S	R	M	R	R	R		
	Nitroxoline (oxyquinolines)	S	M	S	S	S	M	S	S		
	Rifampicin	M	S	S	S	S	R	S	S		
	Levomecetin	S	S	S	S	S	R	M	M		
	Fluoroquinolones										
I	Nalidixic acid	R	R	R	R	R	R	R	R		
	Ofloxacin	R	M	M	M	R	R	M	M		
II											

Table 1 (continued)

Mechanism of action		Group of antibiotic		Strain of bacteria									
Name		Generation		<i>B. animalis</i> VKL	<i>B. animalis</i> VKB	<i>L. acidophilus</i> IMV B-7279	<i>L. casei</i> IMV B-7280	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281	<i>L. rhamnosus</i> LB-3 VK6	<i>L. plantarum</i> LM VK7	<i>L. delbrueckii</i> LE VK8		
Ciprofloxacin				R	R	S	M	R	R	M	M		
Norfloxacin				R	M	R	M	M	R	M	R		
Levofloxacin		III		S	R	R	M	R	R	M	M		
Sparfloxacin				S	S	S	R	R	R	M	M		
Pefloxacin		II		M	M	M	M	R	M	M	M		

Antibiotic resistance/diameter of growth inhibition zone

S sensitive, M medium resistant, R resistant

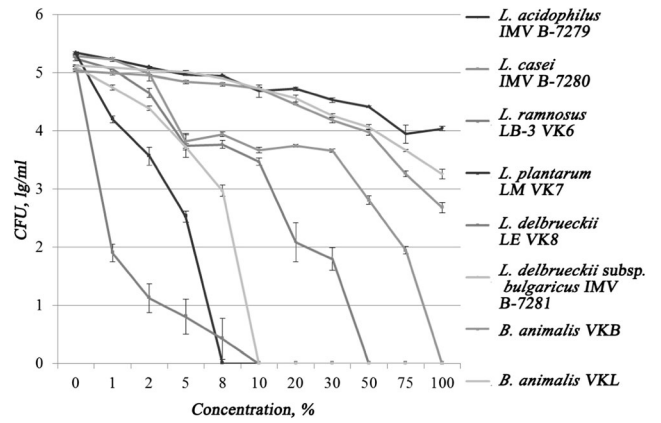


Fig. 2 Resistance of investigated strains to gastric juice

Lactobacillus delbrueckii subsp. *bulgaricus* IMV B-7281 and *L. plantarum* LM VK7 strains had average resistance to bile. *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 strain lost its viability after adding bile into medium at a concentration of 40%. The number of live cells of *L. plantarum* LM VK7 was significantly reduced after strain cultivation in a physiological saline solution with addition of bile in concentrations higher than 10%. The most resistant to bile were *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. rhamnosus* LB-3 VK6, *B. animalis* VKL, and *B. animalis* VKB; their viability was not completely suppressed under the influence of bile in concentrations up to 40% inclusive.

Importantly, that according to our previous studies (unpublished data), *B. animalis* VKL and *B. animalis* VKB strains began to lose their viability after culturing in a medium with bile adding at concentrations of 75 and 50%, respectively.

The results of the study of the resistance of probiotic strains of LAB and bifidobacteria to the action of pancreatin are shown in Fig. 4. All strains tested showed different resistance to pancreatin as demonstrated on Fig. 4. The most susceptible were strains *L. plantarum* LM VK7 and *B. animalis* VKB.

Complete inhibition of *L. plantarum* LM VK7 was observed at a concentration of 4% proteolytic enzymes. *B. animalis* VKB strain lost its vitality at 5% proteolytic

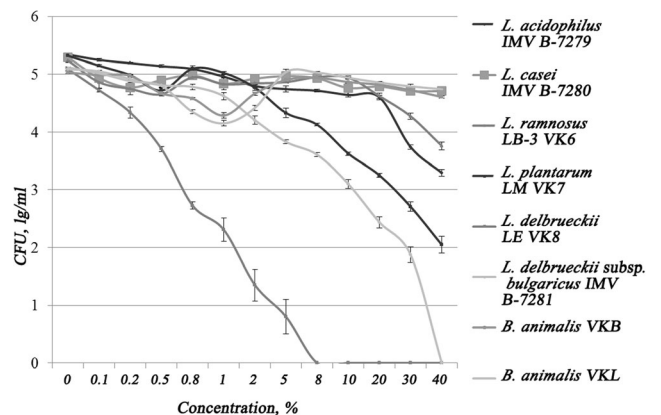


Fig. 3 Strains resistance to bile acids

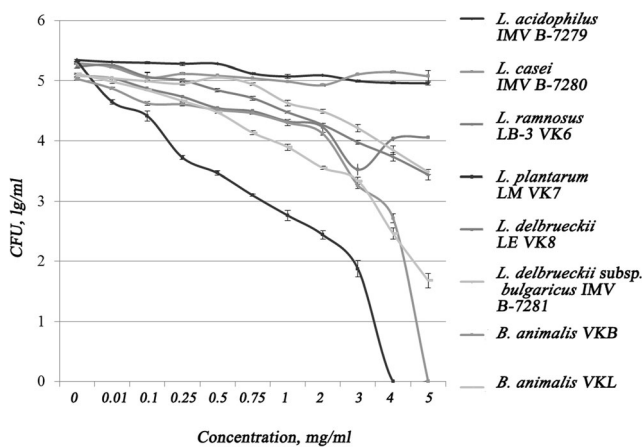


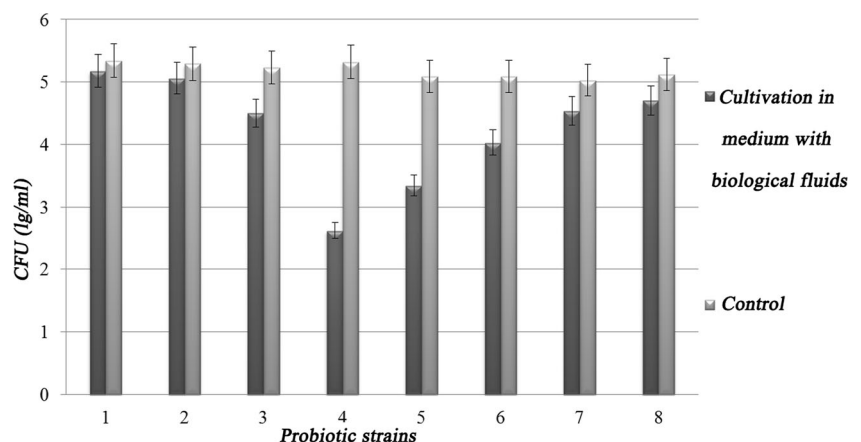
Fig. 4 Resistance of strains to the action of proteolytic enzymes

enzymes. It should be noted that the growth of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *B. animalis* VKL, *B. animalis* VKB, *L. rhamnosus* LB-3 VK6, *L. delbrueckii* LE VK8, and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 was not suppressed completely under the influence of pancreatin in concentrations up to 5% (inclusively).

Since in a living organism probiotics must withstand the effect of these biological fluids in stages, we have investigated their complex effect on the viability of probiotic strains of LAB and bifidobacteria. Since the physiological concentrations of biological fluids are constantly changing and often depend on many factors ranging from food content to human health, we have investigated the phased effects of 2% gastric juice, 1% bile, and 1% proteolytic enzymes.

As presented in Fig. 5, investigated probiotic strains have shown different resistance to the complex influences of biological fluids of the gastrointestinal tract. Thus, the most resistant strains *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *B. animalis* VKB, and *B. animalis* VKL, which survival was more than 90% compared to control (96.96, 95.65, 90.16, 91.9, respectively). Moderate sensitivity to the complex action of

Fig. 5 Stability of probiotic strains to the complex action of biological fluids in the gastrointestinal tract, where 1 = *L. acidophilus* IMV B-7279, 2 = *L. casei* IMV B-7280, 3 = *L. rhamnosus* LB-3 VK6, 4 = *L. plantarum* LM VK7, 5 = *L. delbrueckii* LE VK8, 6 = *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, 7 = *B. animalis* VKB, and 8 = *B. animalis* VKL



biological fluids in the gastrointestinal tract was noted for strains of *L. rhamnosus* LB-3 VK6, *L. delbrueckii* LE VK8, and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, the survival rate of which was, respectively, 50.00, 86.02, 65.64, and 79.16% compared to control. *L. plantarum* LM VK7 was more sensitive to the complex effects of biological fluids, as its survival rate was 49.25% compared to control.

The ability of LAB and bifidobacteria strains to adhere to epithelial cells in vitro is one of the most important criteria for the selection of potentially probiotic strains for intravaginal use, since it indicates their ability to attach and colonize the vaginal surfaces [18].

Results of study of adhesion of LAB strains *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. rhamnosus* LB-3 VK6, *L. delbrueckii* LE VK8, *L. plantarum* LM VK7, *B. animalis* VKL, and *B. animalis* VKB to the buccal epithelium cells are shown in Table. 2.

The obtained data showed that adhesive properties of the strains according to AIM index were high in *L. casei* IMV B-7280, *B. animalis* VKL, and *B. animalis* VKB; were moderate in *L. delbrueckii* subsp. *bulgaricus* IMV B-7281; and were low in *L. acidophilus* IMV B-7279, *L. rhamnosus* LB-3 VK6, *L. delbrueckii* LE VK8, and *L. plantarum* LM VK7.

According to the AIM, the strains were distributed as follows:

$L. casei$ IMV B-7280 \geq *B. animalis* VKB \geq *B. animalis* VKL \geq *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 \geq *L. acidophilus* IMV B-7279 \geq *L. delbrueckii* LE VK8 \geq *L. rhamnosus* VK6 \geq *L. plantarum* VK7.

Consequently, the investigated LAB and bifidobacteria strains can be used in the production of probiotics for oral use, since they have eliminated the resistance to gastric juice, bile, pancreatic enzymes in physiological concentrations, had the ability to adhere to buccal epithelium cells, and also had different sensitivity to antibiotics of different groups.

Table 2 Adhesive properties of LAB and bifidobacteria strains, $M \pm m$

Strain	Parameter of adhesion		
	AAR, units	PRE, %	AIM, units
<i>L. acidophilus</i> IMV B-7279	2.25 ± 0.10	91.82 ± 2.24	2.45 ± 0.11
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281	1.98 ± 0.06	75.21 ± 4.57	2.64 ± 0.16
<i>L. casei</i> IMV B-7280	6.83 ± 0.27	87.45 ± 3.29	7.81 ± 0.86
<i>B. animalis</i> VKB	4.82 ± 0.42	93.77 ± 2.69	5.14 ± 0.48
<i>B. animalis</i> VKL	4.02 ± 0.53	85.16 ± 4.45	4.72 ± 0.54
<i>L. rhamnosus</i> VK6	1.96 ± 0.17	88.00 ± 4.00	2.23 ± 0.29
<i>L. plantarum</i> VK7	1.88 ± 0.12	88.00 ± 5.00	2.14 ± 0.34
<i>L. delbrueckii</i> LE VK8	2.12 ± 0.19	92.00 ± 3.00	2.30 ± 0.24

According to AIM: 0–1.75 unit = nonadhesive strains, 1.76–2.5 units = low adhesive properties strains, 2.51–4.0 units = moderate adhesive properties, ≥ 4.0 units = high adhesive properties

Discussion

Resistance to antibiotics

Thus, the data received on the antibiotic resistance of individual strains of LAB and bifidobacteria partially coincide with the data of other researchers [30, 31, 47–52]. It has been shown that antibiotic resistant bacteria via food chain could be transmitted from animals to humans [48] and most LAB isolated from humans and farm animals are susceptible to amikacin, ampicillin, first generation of cephalosporins, erythromycin, gentamicin, imipinem, oxacillin, and penicillin. Teuber et al. [47] reported that bifidobacteria have sensitivity to ampicillin, penicillin, cephalosporin, erythromycin, and tetracycline and resistance to vancomycin, gentamicin, and streptomycin. It was also reported that *B. animalis*, *L. delbrueckii* subsp. *bulgaricus*, *L. casei*, and *L. acidophilus* were susceptible to ampicillin, bacitracin, clindamycin, dicloxacillin, erythromycin, novobiocin, and penicillin G and strain-dependent susceptible to cephalothin, chloramphenicol, gentamicin, lincomycin, metronidazole, neomycin, paromomycin, streptomycin, tetracycline, and vancomycin [31]. We have determined that *B. animalis* VKB was susceptible to doxycycline and erythromycin, moderately sensitive to penicillin and canamicin resistant, but it was moderately resistant to ampicillin, penicillin, and tetracycline, and resistant to ofloxacin. Similar patterns were observed with respect to other strains examined. These results have some discrepancies with literature data, since all biological properties are strongly strain-dependent and may have certain differences even among similar strains. Current data have much importance for individualized application of probiotics.

A matter of concern to use resistant to antibiotics strains is that antibiotic resistance per se is not a parameter of safety due to the risk of resistance transfer to pathogenic strains [49–51]. Antibiotic resistant determinants have been previously demonstrated to have the ability to transfer from one *Lactobacillus*

to another and, also from Lactobacilli to other species including pathogens such as *Staphylococcus* [52, 53].

The studied strains meet such important selection criteria as antibiotic resistance according to international guidelines for probiotics like the FAO and WHO [2] and European Food Safety Authority (EFSA) [54, 55].

Resistance to gastric juice

Probiotic strains should have ability to survive the gastrointestinal tract of the host. Such properties as stress response mechanisms and adhesion and colonization factors, as well as by taking advantage of specific energy recruitment pathways increase survival and facilitate to carry out their functional activities [56]. Investigated strains of LAB and bifidobacteria have shown resistance to gastric juice, bile, and pancreatin separately and to their complex action, which is consistent with the results obtained by other researchers. Liu et al. reported that the bifidobacteria strains are resistant to the conditions of the gastrointestinal tract [43]. Chou et al. reported similar results on *L. acidophilus* [44] and noted that their stability depends on the acidity of bile and gastric juice and cultivation time. Consequently, the studied strains tested meet the requirements for probiotics according to this parameter [24, 54, 55].

Resistance to bile acids

Bile tolerance is one of the most crucial properties for probiotic bacteria and among others as it determines its ability to survive in the small intestine and consequently, its capacity to play its functional role as a probiotic [57]. It is not commonly detected, therefore Hassanzadazar et al. reported that among 27 isolated strains, only one, namely *L. casei*, could tolerate acid and bile salt and had antibacterial activity [58].

Recently, the novel modalities for therapeutics intervention targeting the gut microbiome or plasma BAs were reported

[59–61] based on the discovery of metabolic significance of BA signaling. Gut microbiota can alter BA composition contributing to the biotransformation of primary BAs to secondary BAs and to the activation or inhibition of FXR [60].

Gu et al. [61] suggested that gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment via so-called acarbose-gut microbiota-BA axis and distinguished two microbiome clusters (*Bacteroides* and *Prevotella* clusters) interacting with BA metabolism.

Cholesterol metabolism via bile acids interplay with microbiome

Since cholesterol metabolism has strong crosslinks with bile acids circulation according to the “bile salt hydrolase hypothesis” (BSH) and the potential associations between immune modulatory and hypocholesterolemic activity of probiotic strains formulated [8], finding on bile resistance might have a strong input on management patients with cholestasis, atherosclerosis, and associated conditions.

Some strains of LAB and bifidobacteria had effective hypocholesterolemic activity in various models of metabolic diseases and in vitro associated with enzymatic degradation of bile acids, direct binding of cholesterol by cell walls of bacteria changes in expression of several genes involved in lipid metabolism [58, 62, 63]. Almost all bifidobacteria species showed BSH activity, while this activity was detected only in a few species of LAB [62]. In addition, many of the bacterial species of the phylum *Firmicutes* produce butyrate, and a decreased abundance of these bacteria was observed in patients with colorectal cancer [63]. *L. casei* IMV B-7280 (separately) and composition of *B. animalis* VKL/*B. animalis* VKB/*L. casei* IMV B-7280 are effective to decrease cholesterol level, beneficially modulate gut microbiome, and recover the liver morphology in high calorie-induced obesity [8].

Resistance to pancreatic juice

All the tested strains are sensitive to artificial and human pancreatic juice depending on time contact while bifidobacterium strains were found in the study by Del Piano [64] to be more sensitive than LAB strains in particular at higher time contact. No significant difference reported between sensitivity to simulated and human pancreatic juice was reported. Artificial pancreatic fluid is recommended as a standardized, easier, and less costly procedure to test probiotics activity [65]. The prolonged exposures to acid stress is suggested to improve the stability of probiotic strains in the human gut indicating strategy for the production of robust probiotic strains [65].

Adhesiveness, pili

Gram-positive strains of genus *Lactobacillus* have bacterial pili for strengthening adhesion to mucus and protection against stresses of the environment. However, molecular mechanism of mucosal mechanical properties of bacterial pili and their role in immune interaction are still largely unknown [66–69]. Gene cluster *spaCBA*, and the pilus-associated *SpaC* pilin makes possible exertion both long distance and intimate contact with tissue of the host and provides mucus-binding [69]. Gram-positive pili are often modified by sortase-specific cleavage reactions. Glycosylation as a modification of sortase-dependent pili has been reported to play in the immunomodulation of the host by glycoproteins of beneficial [69]. These findings altered vision of underappreciated role of glycoconjugates in bacteria-host interplay. Modification of the complex heterotrimeric pili is of importance for the functional interaction with the host immune lectin receptor DC-SIGN on human dendritic cells [69]. Interaction between pili and whey proteins depends on pH; at acidic pH, pili are immobilized in the collapsed EPS layer; and at neutral pH, pili are easily accessible for interaction with whey proteins [70]. Assessment of probiotic bacteria wall using imaging and molecular modalities is important to evaluate or predict role in adhesion properties and immunomodulatory potential of the strain [39, 71–73]. Growing knowledge on nanomechanics of pili can help to design nanomaterials (potential prebiotics) capable of promote adhesive properties for benefits of bacterial-host interactions [74].

Probiotic gut colonization and biofilm formation and microbiome profiles

Microorganisms are usually studied either in isolation as monoclonal model populations that we manage to grow in the laboratory or highly complex natural communities with influence of number unpredictable factors [75, 76]. Probiotics are able to form complex communities, known as biofilms producing an extracellular polysaccharide matrix, expanding colonization in gut and using adhesion properties of bacteria [77, 78]. This helps to prolong and stabilize their residence in the epithelium and triggering the immune response of the host cell [77]. This facilitate probiotic strains to show their antimicrobial potential against human pathogens [79]. Collectively, the disease protection activity of LAB is correlated with their spatial distribution in the intestinal tissue, with strains showing a balanced distribution (hybrid type) more efficient in protection [80]. Probiotic combinations that inhibit and displace pathogens may be excellent candidates to use in fermented milk products [81]. Using new encapsulation technologies based on ability to biofilms formation allow development of *fourth-generation probiotics* [77].

Gut microbiota being proved with body of evidence to be key players in the regulation of host energy homeostasis and in the pathogenesis of obesity remain limited to be properly studied in clinical set due to effects of number unpredictable and poorly controllable factors in humans [75, 76, 82]. Thus, genetic and environmental factors (diet, drug assumption, lifestyle, and the complex interactions within the complexity of microbiome as a whole till inadequately investigated and can evoke significant inconsistency in the results observed in such trials [8, 82].

Searching for reliable phenotype markers of microbiome relevant for longitudinal observation and reproducible in large population is an essential task for microbiome and probiotic research in clinico.

Recently, *mycobiome* was suggested to have the protective benefits via intestinal colonization by commensal fungi [83, 84] that functionally replace intestinal bacteria in alleviating tissue injury and positively activate protective CD8 T cells. Thus, commensal gut fungi protect local and systemic immunity reactivity by providing tonic microbial stimulation that can functionally replace intestinal bacteria.

Microbial diversity is an important parameter of intestinal health. Thus, lower richness of gut microbiota compositions was found in Western diet consumers shapes the microbial ecosystem [15] and in the populations under the burden of obesity and metabolic disease [85, 86]. Individuals with higher diversity were reported to have a healthier dietary pattern [87, 88]. In a subset from the lower diversity was associated with greater abdominal adiposity according to the TwinsUK cohort study [89]. Variety of metabolites are modulated by the action of gut microbiota richness, number of recently discovered crosslinks between gut microbes, and different circulating metabolites with high predictive and diagnostic potential have been recently identified [90].

Pathologic microbiomes in gut and in distant sites—beneficial modulation by probiotics

In the current research, the probiotic bacteria of LAB and bifidobacteria demonstrated different strain-dependent properties (ability for adhesion and resistance to antibiotics and biological fluids) strongly relevant for their clinical application for effective modulation of microbiota in the gut and in distant sites (e.g., oral, skin, vaginal, and other sites microbiota). This supposes their potential secondary beneficial effects for individualized use in adherence with patient's needs.

Thus, according to their *adhesive* properties according to the AIM, the strains were distributed in the sequence as follows: *L. casei* IMV B-7280 \geq *B. animalis* VKB \geq *B. animalis* VKL \geq *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 \geq *L. acidophilus* IMV B-7279 \geq *L. delbrueckii* LE VK8 \geq *L. rhamnosus* VK 6 \geq *L. plantarum* VK 7. This property provide an opportunity for the bacteria to be fixed on the mucosa

and effectively modulate oral, vaginal microbiome and initiate complex immune response [12], and enhance therapeutic potential for conditions, in which treatment by probiotics is still not supported with significant evidence, like bacterial vaginosis, urinary tract infections, periodontitis, and wound healing, etc. Adhesive properties of strains favor biofilm formation that alter inter-strain interaction; therefore, synergic activity of probiotic strains appear rather speculative. In our previous works and in the literature, only few strain compositions demonstrate specifically higher activity [8] and comparative was not assessed properly up to date.

We hypothesize that composition prescription if necessary should be carefully selected for individual case and recommend to always pick one optimal strain or include as less strains as possible. Effects of separate or combined use with prebiotics should be studied.

The level *tolerance to antibiotics* has importance for probiotic safety considering AMR genes transfer and the concept of “resistome” and also has a potential to stratify strains according to the antibiotic use history during patient's life, and pertinence for infections prevention.

Tolerance to gastric juice and to pancreatic enzymes can provide insights for probiotic pharmacokinetics and development of microencapsulation for probiotic preparations [64], nanomaterials, and potential prebiotics [4] to enhance probiotic function [74], consider conditions like gastritis, hyperacidity on the probiotic efficacy; and on other hand, can suppose treatment modalities for gut diseases like dyspepsia, IBS, IBD, postoperative gut, personalized malnutrition correction, and open prospects for development ferment-guided personalized diets.

These studied *basic* bacterial characteristics can contribute to many *secondary* properties of probiotic species. Thus, relatively *neutral* to trigger biological effects properties like elasticity of bacterial wall can be measured using subcellular imaging [39, 71–73]; in newly discovered strains to predict their probiotic potential and justify stratification strains on potential for immunomodulatory activity [39].

Immunomodulatory properties [5, 7, 39] are hypothesized to be a pillar of clinical effect of beneficial microbes. Development the concept and the term of “immunobiotics”—a probiotics with pronounced immunomodilative activity is a promising avenue for future research [7]. This associated with anti-inflammatory properties [5, 7], treatment broad immune-related pathological conditions; enhancement efficacy of immunotherapy [91], and use of vaccines.

It has been hypothesized that immunomodulatory and *anti-inflammatory properties* [5] likely correlate with secondary clinically relevant effects, in particular the ability to modulate metabolic conditions and to demonstrate *antiobesogenic*, *hypcholesterolemic*, and *liver protective* properties [8]; *hypouricemic* activity has been reported [92] and should be

further studied to provide treatment agenda for patients with gout, also using prebiotics [93].

All probiotic strains demonstrate their primary native anti-bacterial [94], antiviral [95, 96], and antifungal properties [83, 84, 97], and have a clear perspective to be a routine alternative for antibiotics.

Oxygen tolerance of probiotic strains is another essential parameter to be studied in the future. Only a few studies have been done in the field [98, 99]; LAB can potentiate intestinal hypoxia-inducible factor [100]. These data can open new perspectives to manage cases associated with *hypoxia-associated* conditions and stress [100]; develop individualized treatment to patients demonstrating Flammer syndrome phenotype [101]; consider patient stratification on important gut marker as hypoxia signaling, mesenteric ischemia in patients with atherosclerosis [102], and ischemic niches in *cancer genesis* [101].

Potential of probiotics for enhancement safety and efficacy of *regenerative* therapy [103], transplantation stem cells is intriguing challenge to develop hybrid biological therapies.

Finally, many mentioned properties should be implemented to cancer case management as supportive therapy [18] and to facilitate symptoms, associated with treatment [104, 105]. Age and gender aspects are essential issues for selection probiotic species for individual use.

Recommendation for individualized clinical use of probiotics

- Product quality;
- Effectiveness should be proven on the basis of evidence-based medicine for routine use in the clinical setting;
- Personalized (or individualized) approach needed in prescribing probiotic according to the disease, clinical case, and phenotype of the patient;
- Using live microorganisms is essential for therapeutic effect;
- Selecting the “best” strain for particular case (for example, the *L casei* strain has strongest properties in most characteristics);
- The higher effectiveness of *multiprobiotics* has not been proved;
- The dose should be at least 10^9 microbial bodies per 1 ml;
- Use of “prebiotic” substances;
- The appropriate route of delivering a probiotic drug (capsule, gel, novel encapsulation technologies);
- Crucially important is combination with an appropriate diet.

Summarizing data of properties of individual probiotic strains (those studied and augmented with literature data) for translating to the human microbiome working as a whole resulting on host’s health are presented in the Table 3.

Conclusion

We recognized strain-dependent properties of studied LAB and bifidobacteria probiotic strains (adhesive ability, resistance to antibiotics and gut biological fluids) and discussed potential for most effective individualized treatment for gut and distant sites microbiome modulation in adherence with patient’s needs according to host phenotype to obtain the best effect possible. These strains correspond to the probiotic characteristics according to probiotic guidelines, are safe and can be recommended for creation of probiotic preparations for application in humans.

Highlights and recommendations

Current research demonstrated that necessary requirements for the inclusion of studied strains into the composition of probiotic preparations were fully met [2, 54, 55, 65].

- Current research confirms the safety of biological properties of potentially probiotic strains of lactobacilli (LAB) and bifidobacteria;
- Studied strains adhere requirement to probiotic guidelines [2, 54, 55];
- Biological properties of studied bacteria are strain-dependent;
- Individual characteristics provide relevant data for clinical diagnosis and stratification to facilitate individualized/personalized use;
- Other phenotypic changes that require an individual characterization should be studied [65];
- Patient’s phenotype stratification is needed for individualized probiotics use.

We believe that a comprehensive approach for evaluating properties of bacteria to select the “best” probiotic strains for development effective probiotic drug and follow up with further preclinical and clinical studies. Host’s phenotype should be clearly analyzed to suggest a panel of biomarkers for individualized use of probiotics and prebiotics. Some properties demonstrate clear associations among each other with significant correlations [8, 39] and this interplay has high importance for further research. Secondary clinically relevant properties of probiotic treatment can be considered like anti-aging or gut-brain axis effects. Mathematical approach using a variety of algorithmic calculation would provide preparing evidence-supported guideline for prevention and treatment. Considering anticipated rapid microbiome and probiotics research progress within paradigm of predictive preventive personalized medicine, the unification of multidisciplinary approaches is a demand. Thus, as a completion of this new knowledge, open potential road in this direction of creating

Table 3 LAB and bifidobacteria probiotic strains properties and potential secondary effects for beneficial individualized use in adherence with patient's needs

Strain property	Strains with rather low activity	Strains with rather high activity	Host phenotype, clinical condition, potential application
Adhesive properties	According to the AIM, the strains were distributed as follows: <i>L. casei</i> IMV B-7280 \geq <i>B. animalis</i> VKB \geq <i>B. animalis</i> VKL \geq <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281 \geq <i>L. acidophilus</i> IMV B-7279 \geq <i>L. delbrueckii</i> LE VK8 \geq <i>L. rhamnosus</i> VK6 \geq <i>L. plantarum</i> VK7		Distant sites [12] use: oral, vaginal, skin microbiome, wound healing; microencapsulation for probiotic preparations [64]; development of nanomaterials (potential prebiotic [4]) to enhance probiotic function [74]; using subcellular imaging [39, 71–73]; microbiome phenotype (diversity), biofilms
Tolerance to antibiotics	Highly sensitive to the effect of antibiotics that are inhibitors of protein synthesis were strains <i>L. plantarum</i> LM VK7, <i>L. acidophilus</i> IMV B-7279, <i>L. casei</i> IMV B-7280 and <i>B. animalis</i> VKB	–	Probiotic use safety, stratification strains according to antibiotic use history during patient's life, adhere to infection prevention; AMR genes, “resistome”
Tolerance to gastric juice	<i>L. plantarum</i> LM VK7	<i>L. acidophilus</i> IMV B-7279, <i>L. casei</i> IMV B-7280, <i>B. animalis</i> VKB, <i>B. animalis</i> VKL	Gastritis, microencapsulation is the future of probiotic preparations [64]
Tolerance to bile	The most susceptible were strains <i>L. plantarum</i> LM VK7 and <i>B. animalis</i> VKB. Complete growth inhibition of <i>L. plantarum</i> LM VK7 was observed at a concentration of 4% proteolytic enzymes. <i>B. animalis</i> VKB strain lost its vitality at 5% proteolytic enzymes	The growth of <i>L. acidophilus</i> IMV B-7279, <i>L. casei</i> IMV B-7280, <i>B. animalis</i> VKL, <i>B. animalis</i> VKB, <i>L. rhamnosus</i> LB-3 VK6, <i>L. delbrueckii</i> LE VK8, and <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281 was not suppressed completely under the influence of pancreatin in concentrations up to 5% (inclusive)	Cholestasis; BA-associated diseases, cholesterol metabolism
Tolerance to pancreatic enzymes	Bifidobacterium strains was reported to be more sensitive than LAB strains [64]	Strains <i>L. acidophilus</i> IMV B-7279, <i>L. casei</i> IMV B-7280, <i>B. animalis</i> VKL, <i>B. animalis</i> VKB, <i>L. rhamnosus</i> LB-3 VK6, <i>L. delbrueckii</i> LE VK8, and <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281 were resistant to pancreatic enzymes	Dyspepsia, IBS, IBD, postoperative gut; personalized malnutrition correction; prospects for development ferment-guided personalized diets
Elasticity of wall [39]	The rigidity of the cell walls among LAB was distributed as follows: <i>L. acidophilus</i> IMV B-7279 > <i>L. casei</i> IMV B-7280 > <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281; among the strains of bifidobacteria: <i>B. animalis</i> VKB > <i>B. animalis</i> VKL		Stratification strains on potential for immunomodulatory activity
Immunomodulatory properties [5, 7, 39]	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281, <i>B. animalis</i> VKL, and <i>B. animalis</i> VKB	<i>L. acidophilus</i> IMV B-7279 or <i>L. casei</i> IMV B-7280	Development immunobiotics [7] broad immune-related pathological states; enhance immunotherapy [91]
Anti-inflammatory properties [5]		Variety of bifidobacterium and LAB strains	Clostridial infection, respiratory infections (evidence-supported), vaginal infection, dysbiosis, visceral pain, cancer, etc.
Antiobesogenic [8]	<i>B. animalis</i> VKB/ <i>B. animalis</i> VKL	<i>L. casei</i> IMV B-7280, <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281, <i>B. animalis</i> VKB, <i>B. animalis</i> VKL (separately) or <i>B. animalis</i> VKL/ <i>B. animalis</i> VKB/ <i>L. casei</i> IMV B-7280, and <i>L. casei</i> IMV B-7280/ <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281	Metabolic syndrome (MetS) management
Hypocholesterolemic properties [8]	<i>B. animalis</i> VKL and <i>B. animalis</i> VKB	<i>L. casei</i> IMV B-7280 and <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> . Nearly all bifidobacteria strains but only few species of LAB showed BSH activity [62].	Cardio-vascular diseases, MetS

Table 3 (continued)

Strain property	Strains with rather low activity	Strains with rather high activity	Host phenotype, clinical condition, potential application
Liver protective properties [8]	<i>B. animalis</i> VKB or <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281 (separately) and <i>B. animalis</i> VKL/ <i>B. animalis</i> VKB	<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> IMV B-7281, <i>B. animalis</i> VKB	Liver fibrosis, cancer
Hypouricemic properties [92]*		<i>B. longum</i> 51A	Gout; use of prebiotics (phenugreek [93])
Effects with prebiotics	To be studied—concept has been modified [4]		Create strain-dependent synbiotics and disease-specific for particular conditions (reproductive function [106]), kidney involvement [93])
Antibacterial [94], antiviral [95, 96] properties		<i>L. casei</i> IMV B-7280, <i>L. acidophilus</i> IMV B-7279, <i>B. animalis</i> VKL, and <i>B. animalis</i> VKB have antibacterial and LAB have antiviral properties	Various infections, cancer
Antifungal properties [83, 84, 97]*		<i>L. plantarum</i> [84]	Promising to stratify host's phenotype (to be studied)
Vaccine efficacy enhancement [107, 108]*		<i>L. casei</i> IMV B-7280 –VHB vaccine [107]; <i>B. longum</i> BL999 <i>L. rhamnosus</i> LPR (Hep B vaccine); <i>L. casei</i> GG (LGG)—oral rotavirus vaccine; <i>L. rhamnosus</i> GG—vaccine against H1N1 influenza [108]	Prevention and treatment infectious diseases in particular groups of patients
Oxygen tolerance [98, 99]*	To be studied. Only a few studies have been conducted on the oxygen tolerance of probiotic bacteria. Most of these studies have focused on <i>Bifidobacterium</i> spp. Little is known about the effect of oxygen on the physiology of <i>L. acidophilus</i> <i>L. rhamnosus</i> GG can potentiate intestinal hypoxia-inducible factor [100]		Relevance to hypoxic-conditions, stress; hypoxia-inducible factor [100]; Flammer syndrome phenotype; metastatic cancer [101]; mesenteric ischemia [102]; regenerative treatments [102]
Regenerative therapy [103]*	To be studied		Potential for enhancement safety and efficacy of regenerative therapy [103], transplantation stem cells

*Augmented with literature data

the consolidated model of medical application of probiotics for PPPM as follows.

Personalized and/or individualized approach

“Individual” phenotype of bacterial characteristics considered for most effective individualized treatment via gut, oral, and vaginal and other sites microbiome modulation according to phenotype of the patient is excellent example of *individualized medicine*, while using broad panel of molecular biomarkers and genomic approach (including microbiome obtained in the microbiome-wide association studies) these data will be easily self-translated to “personalized” medical approach for probiotics. Clarification terms “personalized”/“individualized” for probiotics use is important question [13] and also to establish correlations and associations between both approaches.

On the other hand, raising the question for future innovative research needed in direction of cumulating evidence, since, according to Cochrane reviews, the effects of probiotics are considered “evidence-based” only in cases of antibiotic- and *C. difficile*-associated diarrhea and respiratory tract infections [18], the incomplete evidence is a possibly biased by the heterogeneity of trials conducted. Therefore novel PPPM-oriented protocols for a randomized controlled trial using the microbiome-wide association studies on the largest cohorts possible should be developed; e.g., recently protocol was suggested for type 2 diabetes [109]. Probiotic interstrain reciprocity and study of growing panel of potential prebiotics is a great challenge for near future.

Microbiome phenotypes like microbial diversity, fungal (mycobiome) component, and many others are underestimated parameters of *predictive medicine* to recognize predispositions and evaluate treatment responses almost

to any existing disease and number of phenotype markers to be effectively considered during microbiome modulation.

Strong and well-documented *preventive potential* of probiotics includes evidence-supported prevention of respiratory infections and gut disorders [18] and prevention and treatment infectious diseases via enhancement of vaccine use [107, 108].

A clear message sounds from the research to *avoid overusing antibiotics* and broadly propagate the knowledge in this concern. This can reduce the incidence of hospital infections and largely preserve public health.

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Authors' contributions

RVB suggested the idea and design of the study, participated in experiments, did the study analysis, prepared discussion, formulated future outlooks, and performed article drafting. LML and LPB prepared the first draft of manuscript, did the literature analysis, interpreted the results, and performed the statistical analysis. LPB and LML, performed the experiments on animals and participated analysis of the study. VVM performed the experiments. MYS did the organization, revision manuscript, and data interpretation, and contributed to the overall development of the studied topic. All authors read and approved the final manuscript.

Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

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