



The probiotic potential of microorganism strains: A comprehensive study

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Abstract:

Probiotics are live microorganisms that exert beneficial effects on the host by modulating the gut microbiota and synthesizing bioactive compounds. Of particular importance are metabolites involved in energy metabolism, including compounds of the tricarboxylic acid cycle and B vitamins, which act as coenzymes. This necessitates the search for strains with pronounced metabolic activity and resistance to gastrointestinal conditions. We aimed to comprehensively evaluate the *in vitro* probiotic potential of specific microbial strains for their subsequent use in dietary supplements and functional foods.

We studied the following strains: *Propionibacterium troenii* (B-6083, B-6080), *Weissella thailandensis* (B-10412), *Bifidobacterium bifidum* (AC-1779), *Bifidobacterium longum* (AC-1257), *Pediococcus acidilactici* (B-7509, B-7533), and *Enterococcus faecium* (B-4054). The commercial probiotics “Hemopropiovit” and “Bifiform” were used as the controls. The strains’ antibiotic resistance was determined by the disk diffusion method, their survival in the gastrointestinal tract was measured spectrophotometrically, their antagonistic activity was analyzed by the agar diffusion method, while the compositions and contents of microbial metabolites were identified by high-performance liquid chromatography.

The strains were sensitive to antibiotics (inhibition zones > 21 mm), with the highest values recorded for B-6083 (50.6 ± 4.1 mm). High resistance to bile was demonstrated by B-7509 and B-7533 (optical density 2.288 ± 0.049 and 2.364 ± 0.028 at 0.5% bile), as well as B-4054 (optical density 1.242 ± 0.005 at 40.0% bile). The strains AC-1257, B-7509, and B-7533 showed resistance to 6.5% NaCl. The most pronounced antagonistic activity was demonstrated by B-6083 against *Escherichia coli* (inhibition zone 29.47 ± 0.50 mm) and by B-7533 against *Bacillus cereus* (23.07 ± 0.47 mm). The strains exhibited high metabolic activity such as synthesizing bioactive compounds. The highest content of vitamin B₁ was found in B-10412 (23.10 ± 0.44 mg/g), while succinic acid predominated in B-6080 (432.2 ± 8.2 mg/L). The highest total contents of amino acids were observed in B-7509 (145.2 mg/g) and B-10412 (142.1 mg/g). Some strains showed high probiotic potential by demonstrating resistance to stress factors and exhibiting high antagonistic activity. The strains B-7509, B-7533, AC-1257, B-10412, B-4054, B-6083, and B-6080 possess significant probiotic potential and may be considered as promising components for dietary supplements and functional foods. However, further research is needed such as *in vivo* studies in rodents.

Keywords: Probiotics, probiotic potential, lactic acid bacteria, antagonistic activity, resistance to gastrointestinal stress factors, bioactive metabolites

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INTRODUCTION

Probiotics are live microorganisms that provide health benefits if administered in sufficient amounts [1–4]. Their functional value is determined by their complex interactions with the gut microbiota, metabolic pathways, and immune regulatory mechanisms [5–7].

According to modern research, the gut microbiome is key to maintaining homeostasis. Particularly, it influences energy metabolism, the synthesis of bioactive compounds, and the development of the immune response [8–10]. Probiotic microorganisms are therefore an effective tool for maintaining microbial balance and preventing dysbiosis [11–15]. Modern studies of bioac-

tive components in foods have confirmed the role of probiotics in modulating the gut microbiota and regulating inflammatory processes and metabolic disorders [16–18].

Lactic acid bacteria (LAB) are of particular importance among probiotic cultures. These are anaerobic, Gram-positive microorganisms that can grow in the presence of oxygen [19, 20]. Scientists began to take interest in their dietary use at the beginning of the 20th century, when a hypothesis was proposed that fermented dairy products had a positive effect on human health [21].

Currently, LAB are recognized for their role in synthesizing a wide range of compounds that ensure microbiological stability and improve the quality of food products [22, 23]. Most of their functional properties are strain-specific, which necessitates their individual analyses *in vitro* and *in vivo* [24, 25].

LAB of the genera *Lactococcus* [26], *Pediococcus* [27], *Weissella* [28], and *Enterococcus* [29] have common physiological and biochemical characteristics, as well as the capacity for lactic acid fermentation. *Lactococcus* bacteria are traditionally used as a starter culture for intensive lactose fermentation and the formation of desirable sensory characteristics [30]. *Pediococcus* and *Weissella* bacteria have a high technological value and antagonistic activity against foreign microflora due to their ability to produce bacteriocins and exopolysaccharides [27, 31]. Some bacteriocins produced by LAB hold the GRAS status. Therefore, bacteriocins produced by LAB are widely used in food industry and in pharmaceutical industry [32]. Although bacteria of the genus *Enterococcus* also belong to lactic acid bacteria, their use in food and probiotic systems requires strict safety control [33].

Apart from LAB, probiotic compositions can include other bacteria with similar functionalities. For example, members of the genus *Bifidobacterium* are among the most abundant symbionts in the human gut, contributing to the formation of metabolic and immune homeostasis [34]. They produce bioactive compounds that help maintain colonization resistance and reduce the risk of dysbacteriosis [35].

Bacteria of the genus *Propionibacterium* produce propionic and acetic acids, as well as B vitamins [36]. They are used in functional foods due to their metabolic activity and ability to synthesize antimicrobial compounds [37].

The functional activity of probiotic microorganisms is largely determined by the range of metabolites they synthesize. For example, their antimicrobial, anti-inflammatory, and metabolic regulatory activities are due to organic acids, short-chain fatty acids, and other bioactive compounds that they produce [38, 39]. Furthermore, probiotics actively participate in substrate and energy metabolism, creating conditions for gut homeostasis [40, 41].

Organic acids synthesized by probiotic bacteria are particularly important metabolites that regulate microbiota and metabolic processes [42]. When accumulated in large amounts, they decrease the pH and inhibit the growth of opportunistic microorganisms, contributing to the antimicrobial activity of probiotic cultures [43]. In addition, compounds such as citric and succinic acids

are important intermediate metabolites involved in the tricarboxylic acid cycle, which promotes cellular respiration and energy synthesis [44–47].

Amino acids, both essential and nonessential, are equally important metabolites of probiotic bacteria [48–50]. They are involved in maintaining the body's physiological functions and metabolic interactions [51]. Amino acids perform a structural function, regulate immune responses, and act as precursors of bioactive compounds [52].

Probiotic microorganisms also participate in the metabolism of B vitamins. The gut microbiota and probiotic bacteria can synthesize vitamins B₁, B₂, B₃, B₅, B₆, B₉, and B₁₂, which act as coenzymes in key metabolic reactions [53–55]. These vitamins are involved in the metabolism of carbohydrates, fatty acids, and amino acids, as well as in mitochondrial function and cellular energy supply. Participating in cellular respiration, they regulate the Krebs cycle, which supplies the body with energy [56].

In this study, we aimed to assess *in vitro* the probiotic potential of *Lactococcus*, *Pediococcus*, *Weissella*, *Enterococcus*, *Bifidobacterium*, and *Propionibacterium* strains for their subsequent use in dietary supplements and functional foods to prevent metabolic disorders.

STUDY OBJECTS AND METHODS

The following strains were obtained from the All-Russian Collection of Industrial Microorganisms at the State Research Institute of Genetics and Selection of Industrial Microorganisms, the Kurchatov Institute National Research Center (Russia): *Propionibacterium troenii* T-127 (B-6083); *Propionibacterium troenii* T-126 (B-6080); *Weissella thailandensis* A12 (B-10412); *Bifidobacterium bifidum* E319f (AC-1779); *Bifidobacterium longum* U-4 (AC-1257); *Bifidobacterium longum* BGV-11 (AC-1742); *Lactococcus lactis* subsp. *cremoris* 55 K 2-2-2 (B-1689); *Lactococcus lactis* subsp. *cremoris* 791 7 (B-1688); *Pediococcus acidilactici* P-60 (B-7509); *Pediococcus acidilactici* P 60 (B-7533); *Enterococcus faecium* 320 D (B-5000); and *Enterococcus faecium* JDC 86 (B-4054).

The lyophilized cultures were incubated in a sterile solution of 0.9% NaCl for 30 ± 5 min and then transferred onto nutrient media. Strains B-6083, B-6080, B-10412, B-7509, B-7533, and B-5000 were inoculated onto MRS medium, g/L, composed of: 21.5 pancreatic hydrolysate of fish meal with Tween (Dia-M, Russia); 5.0 baker's yeast extract (Dia-M, Russia); 5.0 peptone (Gelatin Peptone, Spain); 20.0 glucose (Pharmstandard Leksredstva, Russia); 2.0 KH₂PO₄ (Chem-ex, Russia); 3.0 sodium acetate (Chem-ex, Russia); 2.0 ammonium citrate dibasic (Chem-ex, Russia); 0.1 MgSO₄ × 7H₂O (Chem-ex, Russia); and 0.05 MnCl₂ × 4H₂O (Chem-ex, Russia). Strains AC-1779, AC-1257, AC-1742, B-1689, B-1688, and B-4054 were inoculated onto Bifidum medium, g/L, composed of: 30.0 pancreatic casein hydrolysate (Dia-M, Russia); 5.0 baker's yeast extract; 7.5 glucose; 2.5 lactose (Chem-ex, Russia); 0.5 cysteine (Dia-M, Russia); 2.5 NaCl (REAKHIM, Russia); 0.5 MgSO₄ × 7H₂O; 0.5 ascorbic acid (Dia-M, Russia); and 0.3 sodium acetate. The strains grown on the nutrient media were stored at 4 ± 1°C.

The following commercial probiotic preparations were purchased from the Edelweiss-O pharmacy (Russia) to be used as positive controls:

- Hemopropiovit (control 1), which contains *Propionibacterium freudenreichii* III85, *Bifidobacterium bifidum* 83, and *Lactococcus lactis* subsp. *cremoris* 244;
- Biform (control 2), which contains *Enterococcus faecium* and *Bifidobacterium longum*.

Cultural characterization. For cultural characterization, daily cultures were pre-grown on MRS or Bifidum agar nutrient media. Bacterial strain suspensions were surface-seeded onto the media with a Drygalski spatula. Their morphological characteristics were assessed after Gram staining [57] using a Micro-GRAM-NITsF reagent kit (MiniMed, Russia) and an AxioScope A1 upright microscope (Carl Zeiss AG, Germany).

The cultures were further cultivated at $37 \pm 2^\circ\text{C}$ to imitate the physiological conditions of the human gastrointestinal tract and identify those strains which could be adapted to *in vivo* conditions [58].

Preparation of bacterial suspensions. Microbial strains were plated on MRS or Bifidum solid nutrient media. Daily cultures were cultivated in a TV-80-1 thermostat (Ryazan Instrument-Making Plant, Russia). The microbial suspensions were adjusted to a density of 0.5 on the McFarland scale in the corresponding liquid media using a Densichek Plus densitometer (BioMerieux, France). Strains from passages 3–4 were used in further studies.

Antibiotic resistance. Antibiotic resistance of the strains was assessed by the disk diffusion method [59]. For this, 100 μL of a bacterial suspension was inoculated onto the surface of the agar medium. Then, disks were placed with azithromycin (15 μg), cephalexin (30 μg), gentamicin (120 μg), streptomycin (300 μg), and tetracycline (30 μg) (Bio-Rad, France). Disks soaked in distilled water were used as a negative control. After 24 ± 1 h of incubation, the diameters of the inhibition zones were measured and the strains were classified as resistant (< 9.9 mm), intermediate (10.0–20.9 mm), or sensitive (> 21.0 mm).

Resistance to gastrointestinal conditions. Resistance to bile, salt, and stressful pH conditions was determined spectrophotometrically at 600 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Japan) [60]. Resistance to bile (0.5, 20.0, and 40.0% of the medium volume) was determined as described by Kitaevskaya [61]. Resistance to elevated salt (NaCl) concentrations (2.0, 4.0, and 6.5% of the medium volume) and to stressful pH conditions (2.0, 3.0, 5.0, and 9.2) was determined as described in General Pharmacopoeia Monograph 1.7.2.0012.15, “Industrial probiotic strains and probiotic control strains”. Optical density was measured relative to the nutrient medium without a microbial suspension. Samples of nutrient media inoculated with microbial suspensions were used as negative controls.

Antimicrobial activity of bacterial metabolites. Antagonistic activity of microbial metabolites was determined by the agar diffusion method as described by

Davoodabadi *et al.* [62] and Pazhoohan *et al.* [63]. The strains obtained from the All-Russian Collection of Industrial Microorganisms (Russia) were used as test cultures, namely: *Escherichia coli* (B-6645); *Pseudomonas aeruginosa* (B-8243); *Bacillus cereus* (B-8076); *Enterococcus faecalis* (B-8173); *Klebsiella pneumoniae* (B-7001).

For this, 300 μL of a suspension of test cultures (0.5 McFarland turbidity) was inoculated onto a solid nutrient medium (MPA), g/L, composed of: 10.0 peptone; 11.0 meat extract (HiMedia, India); 5.0 NaCl; and 20.0 bacteriological agar (HiMedia, India). Wells with a diameter of 6–8 mm were filled with 50 μL of the culture broth with microbial metabolites, while the control well was filled with 50 μL of distilled water (negative control). A disk with an antibiotic (positive control) was placed in the center of the plate, namely: streptomycin (300 mg), cephalexin (30 μg), gentamicin (120 μg), and tetracycline (30 mg). The plates were kept at room temperature ($22 \pm 2^\circ\text{C}$) for 1–2 h and then incubated at $37 \pm 2^\circ\text{C}$ for 16–18 h. Antagonist activity was determined by measuring the inhibition zones and classified as “no activity” (< 10.9 mm), “weak” (11.0–16.9 mm), “moderate” (17.0–22.9 mm), or “high” (> 23.0 mm) [64].

To obtain microbial metabolites, a microbial suspension (10% of the medium volume) was added to centrifuge tubes (Eppendorf, Germany) containing the nutrient medium and cultivated under steady-state conditions in a TV-80-1 thermostat. The biomass was separated from the culture broth at 7,000 g in a 5430 centrifuge (Eppendorf, Germany). The culture broth was subsequently tested for antagonist activity and biocompatibility.

Microbial growth curves. Microbial growth curves were obtained to identify points of exponential growth, stationary growth, and the death phase and to study the qualitative and quantitative composition of metabolites at these points. The growth curves were obtained spectrophotometrically at 600 nm in real time using an RTS-8 Plus bioreactor (Biosan, Latvia) [65]. For this, a bacterial suspension was inoculated into liquid MRS or Bifidum media (5% of the medium volume) and cultivated under stationary conditions until the death phase was reached.

Quantitative determination of metabolites. The composition of metabolites was determined by high-performance liquid chromatography (HPLC) on a Shimadzu LC-20 Prominence chromatograph (Shimadzu Corporation, Japan) with a Shimadzu SPD20MA diode array detector and a Phenomenex Gemini 5 μm C-18, 250 $\times$ 4.6 mm column. Water-soluble vitamins were determined according to State Standard R 55482-2013 and as described by Frolova *et al.* [66]. Organic acids were determined according to State Standard 33410-2015. Amino acids were determined by reversed-phase HPLC as described by Belashova *et al.* [67].

The microbial strains were cultivated under stationary conditions and sampled at three growth points: exponential, stationary, and death phases. The samples were analyzed for water-soluble B vitamins (B_1 , B_2 , B_3 , B_5 , B_6 , and B_{12}), amino acids (tryptophan, glutamic acid,

serine, glycine, histidine, glutamine, arginine, lysine, threonine, alanine, proline, asparagine, tyrosine, methionine, valine, cysteine, cystine, leucine, isoleucine, and phenylalanine), and organic acids (oxalic, tartaric, maleic, citric, and succinic acids).

Biocompatibility of the strains. Biocompatibility was assessed by using the agar diffusion method with some modification [68]. A microbial suspension was uniformly applied to MRS or Bifidum agar media. Then, 50 µL of the culture broth was placed into wells with a diameter of 6–8 mm. The plates were kept at $22 \pm 2^\circ\text{C}$ for 1–2 h and then incubated at $37 \pm 2^\circ\text{C}$ for 16–18 h. Strain biocompatibility was assessed by measuring the growth inhibition zone: growth inhibition (> 10.0 mm; indicated as “–”); biocompatibility (< 10.0 mm with no signs of growth stimulation; indicated as “+”); and growth stimulation (< 10.0 mm with signs of growth stimulation; indicated as “++”).

The assays were performed in triplicate and the results were presented as a mean and standard error.

RESULTS AND DISCUSSION

Cultural characterization. Since the strains were obtained from the All-Russian Collection of Industrial Microorganisms, their taxonomic affiliation and safety needed no confirmation. Based on Gram staining, the strains were characterized as Gram-positive bacteria.

Cultural and morphological analysis revealed that the colonies cultivated on agar nutrient media possessed genus-specific characteristics. The colonies had a rounded, convex shape, with smooth edges and a shiny white surface. The colony’s diameter of 1–2 mm was consistent with the standard morphological characteristics of the strains of their genus.

Antibiotic resistance. The European Food Safety Authority and the World Health Organization emphasize in their guidelines that the strains used in the food industry should not contain transferable genes confer-

ring resistance to clinically significant antibiotics. We tested the strains under study for their susceptibility to a range of antibiotics (Table 1).

We found that the susceptibility of microorganisms to antibiotics varied significantly between the bacterial strains and genera. The antibiotic resistance of B-10412 and B-4054 was previously described in [69]. Most of the cultures exhibited high sensitivity to the tested antibiotics, with zones of growth inhibition greater than 21 mm in diameter.

Control 1 demonstrated intermediate sensitivity to many antibiotics. The growth inhibition zones were 10.0–15.3 mm for azithromycin, cephalexin (CXN), gentamicin, and streptomycin (HLS). However, Control 1 was found to be sensitive to tetracycline (TET). In comparison, Control 2 exhibited higher sensitivity to the antibiotics, especially to azithromycin and TET.

The highest sensitivity to azithromycin was found in Strains B-6083, B-6080, B-1688, and B-1689. Their inhibition zones were 2.0–3.5 times as large as those of Control 1. Strains B-7509 and B-7533 showed smaller growth inhibition zones compared to the other strains. Yet, they were 1.4–2.0 times larger than those of Control 1, but 1.3–1.8 times smaller than those of Control 2. Thus, the cultures retained high sensitivity to macrolide antibiotics, which is consistent with a study of lactic acid bacteria by Duche *et al.* [70].

Most strains were sensitive to cephalexin, with the largest growth inhibition zones (over 50.0 mm in diameter) recorded for the genus *Propionibacterium* (B-6083, B-6080). Strains B-7509 and B-7533, however, showed significantly smaller inhibition zones, indicating their high resistance to β -lactam antibiotics. Given the widespread use of these drugs in veterinary medicine and animal husbandry, the identified sensitivity values can be considered a positive sign, since they demonstrate a lower risk of accumulation and transmission of antibiotic resistance genes through horizontal transfer [71].

Table 1 Effect of antibiotics on the growth of the strains assessed by the disk diffusion method

Sample	Diameter of growth inhibition zone, mm					
	Azithromycin, 15 µg	Cephalexin, 30 µg	Gentamicin, 120 µg	Streptomycin, 300 µg	Tetracycline, 30 µg	Water
B-6083	31.2 ± 0.8	55.4 ± 0.5	39.9 ± 1.0	19.3 ± 3.1	50.6 ± 4.1	0.0 ± 0.0
B-6080	35.6 ± 5.1	54.6 ± 2.5	46.4 ± 0.5	26.5 ± 1.4	45.8 ± 1.6	0.0 ± 0.0
AC-1779	15.9 ± 1.0	36.5 ± 0.5	29.4 ± 0.6	18.6 ± 1.5	19.5 ± 0.5	0.0 ± 0.0
AC-1257	11.7 ± 1.5	34.6 ± 0.6	23.1 ± 2.0	17.1 ± 1.0	15.4 ± 0.5	0.0 ± 0.0
AC-1742	–	–	–	–	–	–
B-1689	21.1 ± 1.0	32.5 ± 0.5	29.5 ± 8.8	29.9 ± 2.0	23.3 ± 0.8	0.0 ± 0.0
B-1688	22.5 ± 0.5	37.4 ± 0.5	22.4 ± 0.5	20.5 ± 0.5	23.5 ± 0.5	0.0 ± 0.0
B-7509	14.2 ± 0.3	10.1 ± 1.0	10.2 ± 0.3	11.4 ± 0.5	17.2 ± 0.9	0.0 ± 0.0
B-7533	19.5 ± 5.2	13.4 ± 1.5	15.1 ± 1.0	9.4 ± 0.5	26.6 ± 5.8	0.0 ± 0.0
B-5000	–	–	–	–	–	–
Control 1 (Hemopropiovit)	10.0 ± 1.0	11.7 ± 2.9	15.3 ± 5.0	15.3 ± 0.6	24.0 ± 2.6	0.0 ± 0.0
Control 2 (Bifiform)	25.7 ± 4.0	14.2 ± 0.3	23.3 ± 3.1	21.3 ± 1.5	29.3 ± 2.1	0.0 ± 0.0

“–” – indicates no growth during the test

Most studied cultures exhibited sensitivity to gentamicin. This antibiotic had the greatest inhibitory effect on Strains B-6083 and B-6080. Their growth inhibition zones were 2.6–3.0 and 1.7–2.0 times as large as those of Control 1 and Control 2, respectively. Strains B-1689, B-1688, and AC-1779 also showed larger (1.5–1.9 times) inhibition zones than those of Control 1. Strains B-7509 and B-7533 demonstrated intermediate resistance. The inhibition of Strain B-7509 was 1.5 times lower than that of Control 1, while this indicator for Strain B-7533 was close to that of Control 1. Resistance to aminoglycosides is natural due to the low permeability of cell membranes and energy metabolism. The significant sensitivity of some strains in our study may indicate the lack of pronounced natural resistance [70].

Streptomycin had the greatest inhibitory effect on Strains B-1689 and B-1688. Strains AC-1779 and AC-1257 showed intermediate resistance, while B-7533 was found highly resistant. This resistance may be associated with the presence of antibiotic resistance genes, such as *aadA*, which encode aminoglycoside enzymes and can be transmitted to other bacteria through horizontal transfer. A number of studies have shown that while adapting to streptomycin, probiotic microorganisms can develop cross-resistance to other antibiotics. This emphasizes the need to evaluate the safety of probiotic strains in terms of the possible spread of resistance genes [72].

Most strains exhibited high sensitivity to tetracycline. The largest zones of growth inhibition were observed for Strains B-6083 and B-6080, with their diameters being 1.9–2.1 and 1.5–1.7 times greater than those for Control 1 and Control 2, respectively. Strains B-1688, B-1689, and B-7533 also showed larger (1.1–1.5 times) inhibition zones than Control 1 and comparable or slightly larger zones than Control 2. In contrast, Strains AC-1779, AC-1257, and B-7509 demonstrated intermediate resistance, with their inhibition zones be-

ing smaller than those for Control 1 (1.2–1.5 times) and Control 2 (1.5–1.7 times). Bacterial resistance to this antibiotic is most often associated with the presence of specific resistance genes, such as *tet(A)*, *tet(L)*, *tet(K)*, and *tet(M)*, which encode mechanisms for active antibiotic efflux from the cell or ribosome protection [73]. Therefore, the intermediate resistance detected in individual strains may indicate potential development of such mechanisms, which requires additional molecular genetic analysis.

Strains AC-1742 and B-5000 did not show any growth on the nutrient media. This could be due to their specific physiological requirements for cultivation conditions.

Resistance to gastrointestinal conditions. An important criterion for selecting a probiotic strain is its survivability in the gastrointestinal tract, specifically its resistance to bile, elevated salt (NaCl) concentrations, and stressful pH conditions. We assessed changes in strain biomass growth in the nutrient media supplemented with varying amounts of bile (Table 2).

The control samples demonstrated high resistance to bile. Specifically, at 0.5% bile, optical density increased 1.1–1.2 times. At higher concentrations (20.0–40.0%), optical density remained at comparable levels or decreased 1.2–1.5 times.

In most cases, adding 0.5% of bile resulted in lower culture growth compared to the control (without bile), but the degree of inhibition varied significantly between the strains. Strains B-7509 and B-7533 demonstrated the highest resistance, which remained steady and only decreased 1.1 times. Their growth was 1.3–1.5 times as high as that of Controls 1 and 2. Strain B-4054 was also resistant, with higher optical density compared to the control. Strains B-6083 and B-6080 showed a 1.9–2.8-fold decrease in growth compared to the control, indicating their sensitivity to bile at 0.5%. Strains AC-1779 and AC-1257 demonstrated higher resistance. Their

Table 2 Effect of bile on strain growth

Sample	Optical density at 600 nm			
	Control (–)	Bile 0.5 %	Bile 20.0 %	Bile 40.0 %
B-6083	1.065 ± 0.004	0.554 ± 0.325	0.335 ± 0.011	0.518 ± 0.012
B-6080	1.143 ± 0.278	0.408 ± 0.070	0.347 ± 0.005	0.461 ± 0.005
B-10412	1.271 ± 0.140	0.784 ± 0.124	0.294 ± 0.001	0.439 ± 0.015
AC-1779	0.700 ± 0.008	0.529 ± 0.053	0.341 ± 0.007	0.468 ± 0.003
AC-1257	1.357 ± 0.027	0.946 ± 0.021	0.311 ± 0.007	0.402 ± 0.002
AC-1742	–	–	–	–
B-1689	0.538 ± 0.002	0.095 ± 0.003	0.242 ± 0.001	0.420 ± 0.013
B-1688	0.491 ± 0.025	0.099 ± 0.003	0.275 ± 0.008	0.462 ± 0.010
B-7509	2.402 ± 0.007	2.288 ± 0.049	0.962 ± 0.163	0.577 ± 0.229
B-7533	2.433 ± 0.001	2.364 ± 0.028	1.362 ± 0.020	0.887 ± 0.051
B-5000	–	–	–	–
B-4054	1.560 ± 0.003	1.568 ± 0.047	0.992 ± 0.067	1.242 ± 0.005
Control 1 (Hemopropiovit)	1.491 ± 0.015	1.710 ± 0.009	1.515 ± 0.019	1.045 ± 0.168
Control 2 (Bifiform)	1.549 ± 0.006	1.625 ± 0.019	1.933 ± 0.087	1.246 ± 0.028

“–” indicates no growth during the test

growth reduced 1.3–1.4 times and was 1.2–1.5 times lower than that of Controls 1 and 2. Strains B-1688 and B-1689 were the most sensitive to bile at 0.5%. Their growth reduced 4.9–5.7 times and was 10–15 times lower than that of Controls 1 and 2.

Increasing the bile concentration to 20.0% caused a more pronounced growth inhibition in most of the cultures. Strains B-6083, B-6080, AC-1257, and B-10412 showed a 3.3–4.3-fold decrease in growth relative to the bile-free control. Their growth was 4.5–6.5 times lower compared to Controls 1 and 2. Strain AC-1779 showed a 2.1-fold decrease in optical density compared to the bile-free control. Strains B-1688 and B-1689 also demonstrated a significant decrease in growth (1.8–2.2 times). Strains B-7509, B-7533, and B-4054 were the most resistant, showing a 2.5, 1.8, and 1.6-fold decrease in growth, respectively. Although their growth was higher than that of most other strains, it was still 1.4–2.0 times lower than that of the controls.

A 40.0% concentration of bile inhibited the growth of most strains, although some cultures maintained relatively high growth rates. Strains B-6083 and B-6080 demonstrated a 2.1–2.5-fold decrease in growth compared to the initial level. Yet, their growth remained higher than that at 20.0% bile, which may indicate partial adaptation. Compared to Controls 1 and 2, their growth was 2.0–2.5 times lower. Strains AC-1779 and AC-1257 showed a 1.5–3.4-fold decrease in growth relative to the initial level. Their growth was 2.5–3.0 times lower compared to the controls. Strains B-1688 and B-1689 showed pronounced sensitivity. Despite a slight increase in growth compared to 20.0% bile, their growth remained 1.02–1.30 times lower than the initial level and 2.5–3.0 times lower compared to the controls. Strains B-7509, B-7533, and B-4054 were the most resistant, with a 4.2, 2.7, and 1.3-fold decrease in growth, respectively, compared to the bile-free control. Their growth rates were closest to those of Controls 1 and 2.

Thus, Strains B-7509 and B-7533 were the most resistant to bile at 0.5–20.0%, while B-4054 exhibited the highest resistance to bile at 40.0%. In some cases, their

growth rates were 3.0–5.0 times as high as those of less resistant strains and were comparable to those of commercial probiotics.

Our results are consistent with those reported by Hu *et al.* [74]. Their study of the *Pediococcus acidilactici* strain revealed a decrease in culture viability with increasing bile salt concentrations. Some cultures maintained high growth rates even at elevated bile concentrations. This indicates the presence of adaptive resistance mechanisms that allow certain strains to overcome the inhibitory effect of bile and maintain their probiotic potential.

Bile has a pronounced antimicrobial effect, disrupting the integrity of cell membranes and causing protein denaturation. Therefore, the ability of bacteria to maintain growth under such conditions is an important indicator of their probiotic potential. Several studies have shown high survival of certain bifidobacteria strains exposed to bile salts. This survival is associated with the development of stable physiological mechanisms for adaptation to these conditions [75].

Probiotic strains must be resistant to elevated salt levels as they pass through the gastrointestinal tract. Therefore, we assessed changes in strain biomass growth in nutrient media with varying NaCl concentrations (Table 3).

At 2.0, 4.0, and 6.5% NaCl, Control 1 showed a 1.3, 1.5, and 1.1-fold decrease in optical density, respectively, compared to its initial level. Control 2 exhibited more stable growth. At 2.0 and 4.0% NaCl, its optical density decreased 1.1-fold, whereas at 6.5%, it decreased 1.5-fold. Thus, Control 2 demonstrated higher resistance to the effects of salt compared to Control 1.

The resistance of Strains B-10412 and B-4054 to NaCl was described in our previous work [69]. At 2.0% NaCl, most of the strains retained their growth, with varying changes in optical density. Strains B-7509 and B-7533 showed virtually no response to salt stress. Their optical density remained at the initial salt-free level (1.1-fold difference) and exceeded the values for Control 1 and 2 (2.1 and 1.7 times, respectively). Strains

Table 3 Effect of elevated salt levels on strain growth

Sample	Optical density at 600 nm			
	Control (–)	NaCl 2.0%	NaCl 4.0%	NaCl 6.5%
B-6083	1.065 ± 0.004	0.691 ± 0.029	0.480 ± 0.008	0.340 ± 0.008
B-6080	1.143 ± 0.278	1.030 ± 0.034	1.083 ± 0.057	0.635 ± 0.051
AC-1779	0.700 ± 0.008	0.688 ± 0.012	0.904 ± 0.057	0.713 ± 0.140
AC-1257	1.357 ± 0.027	1.394 ± 0.073	1.392 ± 0.022	1.397 ± 0.064
AC-1742	–	–	–	–
B-1689	0.538 ± 0.002	0.433 ± 0.011	0.229 ± 0.049	0.117 ± 0.004
B-1688	0.491 ± 0.025	0.675 ± 0.032	0.718 ± 0.011	0.360 ± 0.209
B7509	2.402 ± 0.007	2.412 ± 0.020	2.189 ± 0.002	1.713 ± 0.009
B7533	2.433 ± 0.001	2.419 ± 0.004	2.157 ± 0.002	0.910 ± 0.040
B-5000	–	–	–	–
Control 1 (Hemopropiovit)	1.491 ± 0.015	1.130 ± 0.168	0.962 ± 0.010	1.390 ± 0.355
Control 2 (Bifiform)	1.549 ± 0.006	1.444 ± 0.013	1.358 ± 0.012	1.057 ± 0.095

“–” – indicates no growth during the test

AC-1257 and B-6080 also demonstrated high resistance. Their optical density was comparable with the initial level (1.1-fold difference) and was 1.2–1.3 times as high as that for Control 1. Strain AC-1779 showed virtually no change in growth, but its optical density was 1.6 and 2.1 times lower than that for Control 1 and 2, respectively. Strain B-6083 had a 1.5-fold decrease in growth and was inferior to Control 1 and 2 (1.6 and 2.1 times, respectively). Strain B-1689 was the most sensitive, its optical density being 1.2 times lower than the initial level, as well as 2.6 and 3.3 times lower than the values for Control 1 and 2, respectively.

At 4.0% NaCl, most strains retained their resistance, but some had more pronounced changes. Strains B-7509 and B-7533 remained highly resistant, with a 1.1-fold decrease in optical density and higher values compared to Control 1 and 2 (2.3 and 1.6 times, respectively). Strain AC-1257 demonstrated stable growth, 1.4 times the value for Control 1. The growth of Strain B-6080 remained at the initial level and was comparable with Control 2. Strain AC-1779 showed a 1.3-fold increase in optical density over the initial level, but remained 1.5 times lower than Control 2. Strain B-6083 had a 2.2-fold decrease in growth, its optical density being 2.0 and 2.8 times lower than Control 1 and 2, respectively. The most pronounced growth inhibition was observed in Strain B-1689, with a 2.3-fold decrease and optical density being 4.2 times and almost 6.0 times lower than the values for Control 1 and 2, respectively.

At 6.5% NaCl, some of the strains showed a more pronounced decrease in growth, while others retained a relatively high resistance. Strain AC-1257 maintained high growth, its optical density remaining virtually unchanged at 1.3 times over Control 2. Strain B-7509 showed a 1.4-fold decrease, but its optical density was 1.2 and 1.6 times as high as the values for Control and 2, respectively. Strain B-7533 had a more pronounced decrease (2.7 times), but its optical density was 1.2

and 1.5 times lower than Control 2 and 1, respectively. Strain B-6080 showed a 1.8-fold decrease in growth and its optical density was 2.2 and 1.7 times lower than that of Control 1 and 2, respectively. Strain AC-1779 showed virtually no decrease in growth, but its optical density was 2.0 and 1.5 times lower than that of Control 1 and 2, respectively. Strain B-6083 demonstrated a 3.1-fold decrease in growth, with its optical density being 4.1 and 3.1 times lower than that of Control 1 and 2, respectively. Strain B-1689 was the most sensitive, with its optical density decreasing 4.6-fold, which was 11.9 and 9.0 times lower than the values for Control 1 and 2, respectively.

Thus, according to the results, Strains AC-1257, B-7509, and B-7533 demonstrated the greatest resistance to elevated salt levels and maintained high growth at 6.5% NaCl. Meanwhile, Strain B-1689 was the most sensitive to salt stress. Overall, the growth rates of several cultures under study were comparable to those for the control probiotic preparations.

Our results are consistent with the studies reporting that lactic acid bacteria can tolerate elevated concentrations of NaCl and maintain growth at 6.5% NaCl. Madushanka *et al.* [76] found that most isolates of lactic acid bacteria retained growth at NaCl concentrations of up to 6.5% due to their physiological and biochemical mechanisms of adaptation to osmotic stress. Nidamarthi *et al.* [77] demonstrated that probiotic strains were able to maintain growth at up to 6.0% NaCl, but then moderately decreased growth at higher concentrations.

Probiotic strains must maintain their viability under stressful pH conditions as they pass through the gastrointestinal tract. Therefore, we assessed their biomass growth in nutrient media with pH values of 2.0, 3.0, 5.0, and 9.2 (Table 4).

The acidic and alkaline environments had a pronounced effect on the growth of the strains under stressful pH conditions, with varying degrees of resistance.

Table 4 Effect of stressful pH conditions on strain growth

Sample	Optical density at 600 nm				
	Control (–)	pH 2.0	pH 3.0	pH 5.0	pH 9.2
B-6083	1.065 ± 0.004	0.180 ± 0.001	0.202 ± 0.008	0.173 ± 0.001	1.761 ± 0.041
B-6080	1.143 ± 0.278	0.155 ± 0.001	0.169 ± 0.007	0.154 ± 0.002	1.269 ± 0.156
B-10412	1.271 ± 0.140	0.157 ± 0.001	0.285 ± 0.135	0.113 ± 0.001	1.373 ± 0.157
AC-1779	0.700 ± 0.008	0.079 ± 0.002	0.067 ± 0.001	0.250 ± 0.005	0.328 ± 0.090
AC-1257	1.357 ± 0.027	0.077 ± 0.001	0.074 ± 0.001	0.336 ± 0.010	1.050 ± 0.034
AC-1742	–	–	–	–	–
B-1689	0.538 ± 0.002	0.075 ± 0.001	0.067 ± 0.001	0.137 ± 0.003	0.187 ± 0.009
B-1688	0.491 ± 0.025	0.072 ± 0.001	0.068 ± 0.001	0.219 ± 0.016	0.587 ± 0.046
B-7509	2.402 ± 0.007	0.178 ± 0.008	0.167 ± 0.005	2.364 ± 0.003	0.426 ± 0.021
B-7533	2.433 ± 0.001	0.187 ± 0.002	0.187 ± 0.002	2.387 ± 0.006	0.432 ± 0.021
B-5000	–	–	–	–	–
B-4054	1.560 ± 0.003	0.187 ± 0.002	0.160 ± 0.002	0.877 ± 0.067	1.959 ± 0.037
Control 1 (Hemopropiovit)	1.491 ± 0.015	0.112 ± 0.001	0.112 ± 0.003	0.987 ± 0.008	1.774 ± 0.114
Control 2 (Bifiform)	1.549 ± 0.006	0.110 ± 0.002	0.106 ± 0.006	0.692 ± 0.033	1.845 ± 0.006

“–” – indicates no growth during the test

At pH 2.0, no growth was observed of any of the strains under study compared to the control. The optical density of Strains B-6083 and B-6080 decreased 5.9 and 7.4 times, respectively. For Strain B-10412, this indicator decreased 8.1-fold. Bifidobacteria strains (AC-1779 and AC-1257) were more sensitive, with their optical density decreasing 8.9 and 17.6 times, respectively. Strains B-1689 and B-1688 showed a 7.2–7.5-fold decrease in optical density. Strains B-7509 and B-7533 also showed a significant growth decrease of 13.2 times compared to the control. For Strain B-4054, this indicator decreased 8.3 times. Commercial probiotics (Controls 1 and 2) also exhibited pronounced growth inhibition, with a 13.3–14.1 decrease in optical density.

At pH 3.0, most of the strains showed lower growth, but it was slightly higher in some cultures compared to their growth at pH 2.0. Strains B-6083 and B-6080 had 5.3 and 6.8 times lower optical density, respectively, relative to the control. For strain B-10412, this indicator was higher than at pH 2.0, but it was still 4.5 times lower than the control value. Strains AC-1779 and AC-1257 had a 10.4–18.3-fold decrease in optical density compared to the control. Strains B-1689 and B-1688 demonstrated a decrease of 7.0–7.2 times, while Strains B-7509 and B-7533 showed a decrease of 13.0–14.4 times. Strain B-4054 also had a significant decrease of 9.7 times. The commercial probiotics (Controls 1 and 2) demonstrated comparable sensitivity, with their optical density decreasing 13.3–14.6 times.

At pH 5.0, the strains demonstrated higher growth compared to their rates at pH 2.0 and 3.0. Strains B-7509 and B-7533 were very close to the control values, which indicated their high tolerance to these pH conditions. Strain B-4054 showed a moderate decrease in growth (1.8 times lower than the control). The growth of AC-1779 and AC-1257 remained 2.8–4.0 times lower than that of the control. Strains B-1689 and B-1688 showed a 2.2–3.9-fold decrease. For B-6083, B-6080, and B-10412, the growth rates remained significantly (6.2–11.2 times) lower than the control. The commercial probiotics demonstrated a 1.5–2.2-fold decrease in growth.

At an alkaline pH (9.2), a number of the strains showed increased growth compared to the control. Strains B-6083, B-10412, and B-4054 had a 1.7, 1.1, and 1.3 times increase in optical density, respectively. The growth of Strain B-6080 was close to the control. Strains AC-1779 and AC-1257 demonstrated a 2.1 and 1.3 times decrease in growth, respectively. Strains B-1689 and B-1688 also showed lower values (2.9 and 1.2 times, respectively) compared to the control. Strains B-7509 and B-7533 showed a marked decrease (5.6 times) in growth relative to the control. The commercial probiotics, on the contrary, demonstrated a 1.2-fold increase in optical density.

Thus, we found that most of the strains were sensitive to highly acidic pH values (2.0–3.0), while some cultures maintained high growth in moderately acidic (pH 5.0) and alkaline (pH 9.2) environments. Strains

B-7509 and B-7533 were the most resistant at pH 5.0, while Strains B-4054 and B-6083 were the most resistant at pH 9.2. In some cases, the growth rates of the cultures were comparable to those for the commercial probiotics.

According to literature, probiotic microorganisms should remain viable after passing through the gastrointestinal tract at low pH values of 2.0–6.5 [78, 79]. Our results demonstrate that some of the cultures can maintain their viability under stressful pH conditions. This is an important indicator of their potential adaptation to the conditions in the upper gastrointestinal tract.

Our results are consistent with the studies on lactic acid bacteria exposed to neutral and alkaline pH values. Several studies have shown that lactic acid bacteria isolates can grow at pH 7.0–9.0, although their growth intensity may vary between the strains [80]. Certain cultures maintained or even increased growth at pH 9.2, indicating the presence of mechanisms for adapting to alkaline stress.

Antimicrobial activity. We assessed the antagonistic activity of the probiotic strains against the test strains of pathogenic and opportunistic microorganisms (Table 5).

Control 1 exhibited weak antagonistic activity against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* and no activity against *Escherichia coli* and *Bacillus cereus*. Control 2 demonstrated moderate activity against *K. pneumoniae*, weak activity against *E. coli*, *B. cereus*, and *E. faecalis*, and no activity against *P. aeruginosa*.

The resistance of Strains B-10412 and B-4054 to *E. coli* is described in our previous work [68]. Strain B-6083 exhibited the most pronounced antagonistic activity against *E. coli*. It was 2.4 times higher than the activity of Control 2 and over 2.4 times higher than that of HLS. Strains AC-1779 and AC-1257 also demonstrated high activity, 1.9 and 2.0 times higher than the values for Control 2 and HLS. Strains B-10412 [69] and B-7533 demonstrated weak activity, at the level of Control 2 and HLS. The remaining strains showed no antagonistic activity against *E. coli*.

Antagonistic activity against *P. aeruginosa* was moderate in Strain AC-1779: it was 1.4 times higher than in Control 1 but 1.2 times lower than in gentamicin. Strains AC-1257, B-7509, B-7533, and B-10412 exhibited weak activity: it was comparable to Control 1, but significantly (1.5 times or more) lower than in gentamicin. The remaining strains showed no activity against *P. aeruginosa*.

Antagonistic activity against *B. cereus* was the most pronounced in Strain B-7533, 1.5 times the value for Control 2 but almost the same (1.1-fold difference or less) as CXN. Strain B-7509 demonstrated moderate activity, 1.3 times higher than Control 2 but 1.1 lower than CXN. Strains AC-1779, AC-1257, B-10412, B-1689, B-1688, and B-4054 showed weak activity. Strains B-6083 and B-6080 were completely inactive.

Table 5 Antagonistic activity of the probiotic strains

Sample	Diameter of growth inhibition zone, mm				
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>	<i>Enterococcus faecalis</i>	<i>Klebsiella pneumoniae</i>
B-6083	29.47 ± 0.50	0.00 ± 0.00	10.05 ± 0.20	11.16 ± 0.22	0.00 ± 0.00
B-6080	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
B-10412	*	13.26 ± 0.27	14.55 ± 0.30	10.43 ± 0.21	15.67 ± 0.32
AC-1779	23.53 ± 0.50	17.64 ± 0.36	16.94 ± 0.33	10.82 ± 0.22	14.28 ± 0.29
AC-1257	23.60 ± 0.36	17.18 ± 0.35	13.84 ± 0.26	11.91 ± 0.24	12.09 ± 0.24
AC-1742	—	—	—	—	—
B-1689	0.00 ± 0.00	0.00 ± 0.00	12.40 ± 0.25	13.48 ± 0.27	14.04 ± 0.29
B-1688	0.00 ± 0.00	0.00 ± 0.00	12.72 ± 0.26	15.40 ± 0.31	10.58 ± 0.22
B-7509	0.00 ± 0.00	13.98 ± 0.29	20.48 ± 0.42	16.38 ± 0.33	14.94 ± 0.30
B-7533	13.23 ± 0.75	13.07 ± 0.27	23.07 ± 0.47	15.81 ± 0.32	19.16 ± 0.39
B-5000	—	—	—	—	—
B-4054	*	0.00 ± 0.00	14.05 ± 0.29	12.71 ± 0.26	15.70 ± 0.32
Control 1 (Hemopropiovit)	0.00 ± 0.00	12.65 ± 0.26	0.00 ± 0.00	9.38 ± 0.19	11.71 ± 0.24
Control 2 (Bifiform)	12.23 ± 0.34	0.00 ± 0.00	15.48 ± 0.32	11.79 ± 0.24	17.90 ± 0.36
Streptomycin, 300 mg	12.00 ± 0.32				
Gentamicin, 120 mcg		20.53 ± 0.33			
Cephalexin, 30 mcg			23.5 ± 0.38		27.6 ± 0.45
Tetracycline, 30 mg				25.5 ± 0.42	
Water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

“—” indicates no growth during the test; * – refers to the authors’ previous publication [68]

Antagonistic activity against *E. faecalis* was the most pronounced in Strains B-7509 and B-7533, 1.7 times higher than in Control 1 and 1.3–1.4 times higher than in Control 2, but 1.6 times lower than in TET. Strains B-1688, B-1689, AC-1257, AC-1779, and B-10412 also showed weak activity, 1.1–1.6 times higher than Control 1 and about the same level as Control 2, but significantly lower (1.6–2.4 times) than TET. Strain B-6083 showed the weakest activity, while B-6080 did not exhibit any inhibitory effect at all.

Antagonistic activity against *K. pneumoniae* was the most pronounced in Strain B-7533. This strain showed moderate values, which were 1.1 times higher than those for Control 2 and 1.6 times higher than those for Control 1, yet 1.4 times lower than those for CXN. Strains B-10412 and B-4054 demonstrated weak activity, 1.3–1.4 times higher than Control 1 and at the same level as Control 2, but 1.7–1.8 times lower than the antibiotic. Strains B-7509, AC-1779, B-1689, and AC-1257 also showed weak activity, 1.1–1.3 times higher than Control 1, but 1.3 times lower than Control 2 and significantly (1.8–2.0 times) lower than CXN. Strains B-6083 and B-6080 showed no activity against *K. pneumoniae*.

Thus, the most pronounced antagonistic activity was observed in B-6083 against *E. coli*, as well as in B-7533 and B-7509 against *B. cereus*, *E. faecalis*, and *K. pneumoniae*. Strains AC-1779 and AC-1257 exhibited high or moderate activity against *E. coli* and *P. aeruginosa*, whereas Strains B-1689, B-1688, and B-4054 demonstrated mostly weak antagonistic activity. In some cases, the strains’ activity was comparable to, or higher than, the activity of commercial probiotics.

Our results are consistent with other studies that report pronounced antimicrobial properties of probiotic microorganisms. Fijan *et al.* [81] studied the antagonistic activity of representatives of the genera *Propionibacterium*, *Bifidobacterium*, *Lactococcus*, and *Enterococcus* against a wide range of opportunistic bacteria, including *E. coli*, *P. aeruginosa*, *E. faecalis*, and *K. pneumoniae*. Their inhibition efficiency varied between the strains and was determined by the production of organic acids, bacteriocins, and other antimicrobial metabolites. Probiotic compositions demonstrated a more pronounced antagonistic effect compared to individual strains, with zones of inhibition of pathogen growth averaging 10.0–20.0 mm. This indicated possible synergism between different probiotic microorganisms. Individual representatives, including propionic acid bacteria, usually exhibited a more moderate inhibitory effect. Sanam *et al.* [82] studied the antagonistic activity of probiotic microorganisms against *E. coli* and *B. cereus*. They found that lactic acid bacteria isolated from mare’s milk can form inhibition zones with a diameter of 29.6 mm for *B. cereus* and 31.2 mm for *E. coli*. The authors assumed that this inhibitory effect was due to the synthesis of various antimicrobial metabolites, including organic acids, diacetyl, hydrogen peroxide, and bacteriocins.

Srinu *et al.* [83] showed that the strains *Bifidobacterium bifidum* and *Bifidobacterium adolescentis* can inhibit the growth of various pathogenic bacteria, with zones of growth inhibition reaching 14.0–18.0 mm in diameter (15.0–18.0 mm for *E. coli* and 14.0–16.0 mm for *K. pneumoniae*). Probiotics of the genus *Pediococcus*

cus also exhibit antagonistic activity. Ouili *et al.* [84] found that the strains of *Pediococcus pentosaceus* isolated from fermented plant products can effectively inhibit the growth of pathogenic microorganisms (*E. coli* and *P. aeruginosa*), with zones of growth inhibition greater than 18.0 mm in diameter. Representatives of the genus *Weissella* can also produce an inhibitory effect on the growth of pathogens, both gram-positive and gram-negative, as reported by Fhoula *et al.* [85]. In particular, they exhibited pronounced antimicrobial activity against *P. aeruginosa*, *E. coli*, and *E. faecalis*, with growth inhibition zones reaching 36.0 ± 1.8 , 26.0 ± 0.9 , and 26.0 ± 1.0 mm in diameter, respectively.

In our study, not all of the cultures exhibited sufficient viability and probiotic potential. Particularly, Strains AC-1742 and B-5000 failed to grow on the nutrient media, possibly due to their specific physiological requirements for cultivation conditions. As a result, we were unable to assess their antagonistic activity and resistance to gastrointestinal stress and excluded them from further analysis.

Strains B-1689 and B-1688 were also excluded from further analysis because of their relatively low resistance to gastrointestinal stress factors. In particular, exposure to bile (0.5%) resulted in significant growth inhibition, with optical density decreasing fivefold compared to the control. At 6.5% NaCl, Strain B-1689 demonstrated the most pronounced decrease in growth among the cultures under study (4.6 times lower than the control). When tested for antagonistic activity, these strains showed no inhibitory effect against *E. coli* and *P. aeruginosa* and demonstrated weak activity against the other pathogen cultures. Taken together, these results indicate a relatively low probiotic potential for these strains.

Microbial growth curves. The microbial growth curves showed changes in the optical density of the strains relative to their cultivation time (Fig. 1).

The data revealed the following growth phases: a lag phase, an exponential phase, a stationary phase, and a death phase. Strains B-7509 and B-7533 began to grow after 4–6 h, indicating a short adaptation period. Strain B-10412 took longer to adapt, its active growth starting after 9 h. Strains AC-1779 and AC-1257 also had quite a long lag phase, with the first noticeable changes recorded between 4 and 6 h. Strains B-6083 and B-6080 had the longest adaptation period, starting to grow after 13 h. In contrast, Strain B-4054 and the control cultures showed growth after 3 h.

The most intense increase in cell numbers was observed between 8 and 22 h of cultivation. Strains B-7509 and B-7533 grew most rapidly, their optical density increasing 4.1-fold between 9 and 23 h. Strain B-10412 showed a slower initial increase, but its optical density increased 8.6-fold between 16 and 24 h. The *Bifidobacterium* strains showed more moderate growth. The optical density of Strains AC-1779 and AC-1257 increased 2.2 and 1.4-fold, respectively, between 15 and 23 h. The *Propionibacterium* strains took longer to start the exponential phase. Strain B-6083 showed a 2.7-fold

growth between 16 and 30 h, while B-6080 showed an 8.5-fold growth between 15 and 30 h. In contrast, Strain B-4054 reached its maximum growth by the 7th h of cultivation and then stopped growing. Controls 1 and 2 also reached the stationary phase quite quickly, within 10 h of cultivation.

The stationary growth phase started after 22–24 h of cultivation for most of the cultures. The highest values were observed in Strains B-7509 and B-7533, with their optical density reaching 7.01 ± 0.15 and 6.61 ± 0.14 , re-

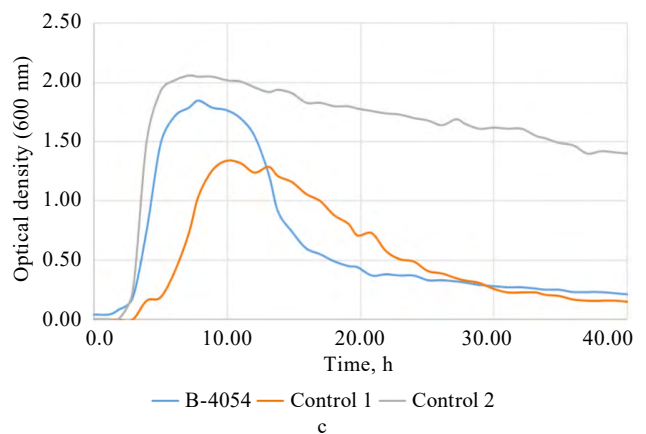
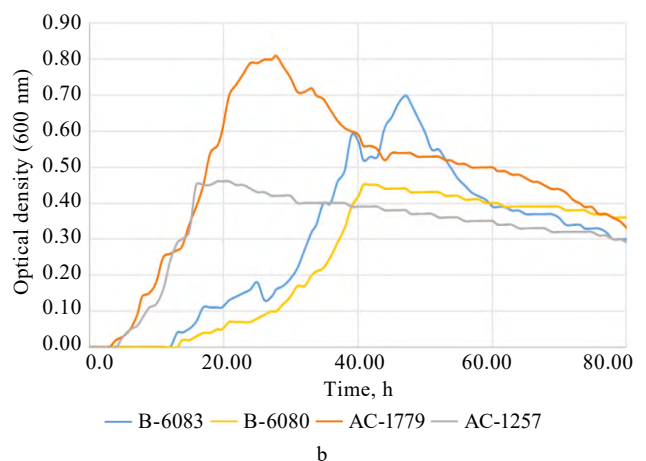
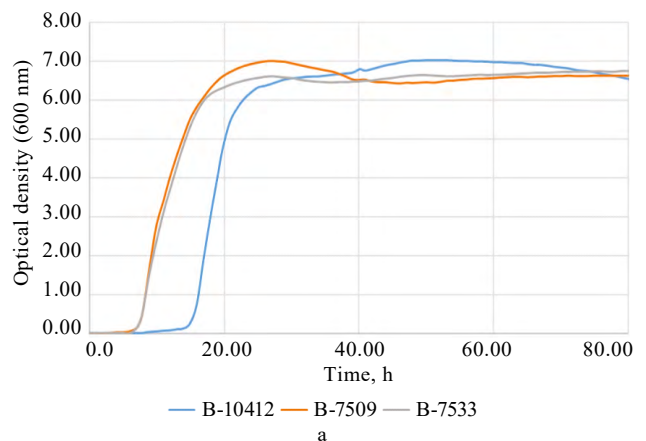


Figure 1 Growth curves for: a – Strains B-10412, B-7509, and B-7533; b – Strains B-6083, B-6080, AC-1779, and AC-1257; and c – B-4054, Controls 1 and 2

spectively, after 27 h of cultivation. Strain B-10412 reached a maximum of 7.03 ± 0.15 after 50 h. The *Bifidobacterium* strains showed significantly low values, reaching their maximum after 28 h (0.81 ± 0.02 for AC-1779) and 19 h (0.46 ± 0.01 for AC-1257), and then stabilizing or gradually decreasing. The *Propionibacterium* strains had even lower maximum values, 0.70 ± 0.01 for B-6083 (47 h) and 0.45 ± 0.01 for B-6080 (41 h). After rapid initial growth, Strain B-4054 entered the stationary phase after 15–16 h, with its values gradually decreasing 3.3-fold by the 45th h of cultivation. The control cultures entered the stationary phase earlier, with Control 1 reaching its maximum after 10 h (1.34 ± 0.03) and Control 2, after 7 h (2.06 ± 0.04).

After reaching their maximum, most cultures showed a gradual decrease in their optical density due to the depletion of nutrients and the accumulation of metabolites. The most pronounced decrease was observed in Strain B-10412 (down to 6.54 ± 0.14 by the 80th h). In contrast, Strains B-7509 and B-7533 hardly decreased at all, their optical density remaining stable throughout the entire observation period. The *Propionibacterium* and *Bifidobacterium* strains showed a more gradual decrease – 2.3-fold for B-6083 between 48 and 80 h, 2.4-fold for AC-1779 between 29 and 80 h, and 1.6-fold for AC-1257 between 22 and 80 h. The optical density of B-4054 and the control cultures decreased much earlier, after 10–15 h of cultivation.

Thus, the growth curves demonstrated that Strains B-7509, B-7533, and B-10412 exhibited the most vigorous growth and a prolonged stationary phase. Strains AC-1779, AC-1257, B-6083, and B-6080 had a longer lag phase and a moderate growth rate. Strain B-4054 and the control cultures showed intermediate growth rates.

Based on the S-shaped growth curves, we identified three time points (1 – exponential phase, 2 – maximum biomass accumulation, and 3 – death phase) at which samples were collected for metabolite analysis (Table 6).

Quantification of microbial metabolites. Table 7 shows the contents of B vitamins in the strains sampled at three time points.

The quantitative analysis of B vitamins revealed that the strains differed significantly in their ability to synthesize vitamins at different stages of growth. Riboflavin (vitamin B₂) was not detected in any of the samples.

Vitamin B₁ had the highest content in Strain B-10412, remaining high throughout the entire cultivation period. In B-6080, its accumulation showed a 6.7-fold increase compared to time point 1. Strains B-7509 and B-7533 increased their thiamine concentrations 1.2 and 3.0-fold, respectively, towards the end of cultivation (time point 3). Strain B-6083 showed a moderate increase at time point 2, followed by a decrease at time point 3. In Strains AC-1779 and AC-1257, thiamine concentrations remained relatively stable between the time points.

Vitamin B₃ had its highest concentrations in the *Bifidobacterium* strains. In AC-1779, the niacin content increased gradually throughout cultivation, while in AC-1257, it increased at time point 2 after a slight decrease. Strain B-10412 showed a noticeable (3.2-fold) decrease in niacin between time points 1 and 2, followed by a partial increase. B-6083 reached a pronounced peak in vitamin B₃ at time point 2, followed by a 2.5 decrease. Strains B-7509 and B-7533 accumulated maximum niacin contents towards the end of cultivation. Particularly, Strain B-7509 had a 2.7-fold increase compared to time point 1. Strain B-4054 showed a sharp decrease in niacin at time point 2, followed by a partial recovery by time point 3.

We found significant fluctuations in vitamin concentrations at individual time points during cultivation. Some strains showed abrupt changes in compounds between the growth phases. For example, B-6080 had a sharp increase in thiamine at time point 2, while B-7533 accumulated the vitamins between time points 1 and 3. Strains AC-1779 and AC-1257 showed more gradual changes (e.g., a gradual increase in niacin).

The contents of vitamins B₅, B₆, and B₁₂ were low in all the strains, less than 0.1 mg/g. However, some cultures consistently produced trace amounts of these compounds. B-10412 showed a gradual increase in vitamin B₆, while B-6080 showed a consistent increase in vitamin B₅ throughout cultivation. Strain B-4054 had 4.5-fold increase in vitamin B₁₂ between time points 1 and 3. In the *Bifidobacterium* strains, pantothenic acid (B₅) and pyridoxine (B₆) were detected at virtually all growth points, indicating high activity of the metabolic pathways.

Table 6 Time points at which samples were collected for metabolite analysis

Strain	Exponential phase, h	Maximum biomass accumulation, h	Death phase, h
B-6083	10.0 ± 1.0	24.0 ± 2.0	40.0 ± 2.0
B-6080	35.0 ± 2.0	41.0 ± 2.0	70.0 ± 2.0
B-10412	16.0 ± 1.0	48.0 ± 2.0	72.0 ± 2.0
AC-1779	16.0 ± 1.0	28.0 ± 2.0	32.0 ± 2.0
AC-1257	12.0 ± 1.0	16.0 ± 1.0	21.0 ± 2.0
B-7509	10.0 ± 1.0	26.0 ± 2.0	35.0 ± 2.0
B-7533	10.0 ± 1.0	26.0 ± 2.0	35.0 ± 2.0
B-4054	4.0 ± 0.5	8.0 ± 1.0	10.0 ± 1.0
Control 1 (Hemopropiovit)	7.0 ± 1.0	10.0 ± 1.0	20.0 ± 1.0
Control 2 (Bifiform)	3.0 ± 0.5	9.0 ± 1.0	14.0 ± 1.0

Table 7 B vitamin contents at all time points

Sample	Time point	Vitamin content, mg/g					
		B ₁	B ₂	B ₃	B ₅	B ₆	B ₁₂
B-6083	1	2.22 ± 0.04	n.d.	1.79 ± 0.03	0	n.d.	0.06 ± 0.01
	2	2.51 ± 0.05	n.d.	5.05 ± 0.10	0	n.d.	0.02 ± 0.01
	3	1.95 ± 0.04	n.d.	2.06 ± 0.04	0	0.02 ± 0.01	0.04 ± 0.01
B-6080	1	2.69 ± 0.05	n.d.	1.71 ± 0.03	0.07 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
	2	17.97 ± 0.34	n.d.	1.39 ± 0.03	0.10 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
	3	2.84 ± 0.05	n.d.	1.43 ± 0.03	0.11 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
B-10412	1	23.10 ± 0.44	n.d.	12.16 ± 0.23	0.12 ± 0.01	0.02 ± 0.01	0.09 ± 0.01
	2	15.88 ± 0.30	n.d.	3.79 ± 0.07	0.10 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
	3	12.32 ± 0.23	n.d.	4.31 ± 0.08	0.10 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
AC-1779	1	1.63 ± 0.03	n.d.	13.54 ± 0.26	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
	2	1.34 ± 0.03	n.d.	13.90 ± 0.26	0.04 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
	3	2.80 ± 0.05	n.d.	14.32 ± 0.27	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
AC-1257	1	1.26 ± 0.02	n.d.	14.48 ± 0.28	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	2	1.44 ± 0.03	n.d.	13.56 ± 0.26	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
	3	1.25 ± 0.02	n.d.	15.82 ± 0.30	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
B-7509	1	12.80 ± 0.24	n.d.	1.29 ± 0.02	0.06 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
	2	—	—	—	—	—	—
	3	15.84 ± 0.30	n.d.	3.53 ± 0.07	0.06 ± 0.01	0.02 ± 0.01	n.d.
B-7533	1	4.82 ± 0.09	n.d.	2.06 ± 0.04	0.05 ± 0.01	0.01 ± 0.01	0.06 ± 0.01
	2	—	—	—	—	—	—
	3	13.75 ± 0.26	n.d.	2.64 ± 0.05	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
B-4054	1	1.67 ± 0.03	n.d.	5.81 ± 0.11	0	n.d.	0.02 ± 0.01
	2	2.32 ± 0.04	n.d.	n.d.	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.01
	3	2.29 ± 0.04	n.d.	1.51 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.09 ± 0.01
Control 1 (Hemopropiovit)	1	3.29 ± 0.06	n.d.	1.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	2	3.55 ± 0.07	n.d.	1.29 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
	3	2.92 ± 0.06	n.d.	9.70 ± 0.18	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Control 2 (Bifiform)	1	2.97 ± 0.06	n.d.	1.62 ± 0.03	0	0.02 ± 0.01	0.02 ± 0.01
	2	2.51 ± 0.05	n.d.	1.83 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.01
	3	1.46 ± 0.03	n.d.	1.23 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01

n.d. – not detected; 0 – detected in a minimum threshold amount; “—” failed analysis; time points: 1 – exponential phase, 2 – maximum biomass accumulation, and 3 – death phase

A number of studies have confirmed the ability of probiotic microorganisms to synthesize B vitamins. These compounds play an important role in energy metabolism by acting as cofactors in enzymatic reactions associated with the formation of ATP [40, 86]. Le-Blanc *et al.* [87] reported that lactic acid bacteria, as well as normal human gut microbiota, are capable of synthesizing thiamine (B₁), riboflavin (B₂), pyridoxine (B₆), folates (B₉), and cobalamin (B₁₂). Probiotic strains *Bifidobacterium longum* and *B. bifidum* secreted thiamine *in vitro*, but failed to produce folates and riboflavin, which indicates their pronounced strain specificity.

Studies also report a possible synergistic interaction between probiotics and B vitamins. For example, Sang *et al.* [88] showed that the combined administration of probiotic strains of the genus *Lactobacillus* and B vitamins in animals improved the absorption of vitamins B₁, B₃, B₅, and B₁₂ by 14.5–71.2% compared to their separate use. The authors assumed that this effect was due to the positive influence of the probiotics on the gut microbiota and vitamin absorption processes, improving their bioavailability.

Our analysis revealed that different strains showed maximum vitamin accumulation at different time points. Furthermore, most of the samples did not demonstrate consistent maximum values at time point 2. This was rather a transitional phase between active growth and a subsequent decline in metabolic activity. Therefore, further research was limited to the comparison between time points 1 (exponential growth phase) and 3 (death phase).

Table 8 presents the contents of organic acids in the strain samples.

Our analysis revealed that the probiotic strains differed significantly in the spectrum and intensity of metabolite synthesis. The most pronounced differences were observed for citric and succinic acids.

The *Propionibacterium* strains showed different metabolic profiles. B-6083 synthesized tartaric, maleic, and citric acids at time point 1, with only a small amount of maleic acid remaining at point 3. B-6080 exhibited a different pattern of metabolic activity. It synthesized significant amounts of succinic and citric acids at time point 1. By time point 3, however, citric acid was

absent, while succinic acid increased 1.2-fold, reaching its maximum accumulation at the death phase.

B-10412 synthesized a wide range of organic acids. At time point 1, this strain contained significant concentrations of citric and succinic acids, along with maleic and tartaric acids. At time point 3, succinic and citric acids decreased 5.5 and 1.5 times, respectively, while tartaric acid increased 3.6 times.

The *Bifidobacterium* strains exhibited significant changes in their metabolic profiles between the time points. Strain AC-1779 showed oxalic, citric, and succinic acids at time point 1, with none of them detected at time point 3. In contrast, AC-1257 synthesized tartaric and succinic acids at time point 1, while oxalic and citric acids were detected at time point 3, with citric acid increasing 13.3-fold.

Strain B-7509 exhibited high concentrations of succinic and citric acids at time point 1, while tartaric and maleic acids were detected at time point 3. Strain B-7533 demonstrated a general increase in most organic acids by time point 3, indicating high metabolic activity during the death phase. In particular, citric acid, succinic acid, and maleic acid increased 2.1, 7.7, and 3.4 times, respectively.

Strain B-4054 had a high concentration of maleic acid at time point 1. At time point 3, maleic acid disappeared, while concentrations of citric, succinic, and tartaric acids increased 3.8, 4.8, and 9.3 times.

The control samples had high concentrations of individual organic acids. Control 1 showed significant amounts of citric, tartaric, and succinic acids at time point 1, which significantly decreased by point 3. Con-

trol 2 exhibited less diversity in its metabolic profile, with citric and succinic acids detected at point 1. At time point 3, tartaric acid remained high, while citric acid decreased.

The synthesis of organic acids is one of the key metabolic functions of probiotic microorganisms. These compounds result from the fermentation of carbohydrates and contribute to the antimicrobial properties of bacteria. The contents of organic acids depend on the strain and its cultivation conditions. Scauro *et al.* [89] reported that the probiotic strains of *Lactiplantibacillus plantarum* synthesized lactic, citric, acetic, ascorbic, malic, tartaric, and other acids. Their accumulation varied significantly between the cultures, indicating pronounced strain-specificity of their metabolism. Ye *et al.* [90] found that probiotic bacteria synthesized a number of organic acids (including succinic and tartaric) and their derivatives involved in the metabolism of the tricarboxylic acid cycle. Increased succinic acid levels are often associated with the activation of metabolic pathways of the Krebs cycle and the conversion of other organic acids during microbial growth. Mohamad *et al.* [91] considered the synthesis of organic acids as one of the main antagonistic activities of probiotic bacteria. They noted that the accumulation of organic acids in the culture medium decreased its pH and created unfavorable conditions for the growth of pathogenic and opportunistic microorganisms. Organic acids such as citric, tartaric, succinic, and others form an acidic environment and inhibit foreign microflora.

Thus, our results confirmed the pronounced strain-specific nature of organic acid synthesis. The

Table 8 Contents of organic acids in the probiotic strains

Strain	Time point	Organic acid content, mg/L				
		Oxalic	Tartaric	Maleic	Citric	Succinic
B-6083	1	n.d.	3.7 ± 0.1	0.6 ± 0.1	15.1 ± 0.3	0
	3	0	0	0.1 ± 0.1	0	0
B-6080	1	0	1.2 ± 0.1	0	25.1 ± 0.5	358.8 ± 6.8
	3	0	3.5 ± 0.1	0	0	432.2 ± 8.2
B-10412	1	0	1.0 ± 0.1	2.2 ± 0.1	59.2 ± 1.1	47.0 ± 0.9
	3	n.d.	3.6 ± 0.1	0.2 ± 0.1	39.6 ± 0.8	8.6 ± 0.2
AC-1779	1	17.7 ± 0.3	0	0	26.3 ± 0.5	8.8 ± 0.2
	3	0	0	0	0	0
AC-1257	1	0	5.8 ± 0.1	0.8 ± 0.1	2.1 ± 0.1	48.2 ± 0.9
	3	16.9 ± 0.3	0	0	27.9 ± 0.5	0
B-7509	1	4.1 ± 0.1	0.9 ± 0.1	0	42.6 ± 0.8	344.3 ± 6.6
	3	0	2.8 ± 0.1	0.9 ± 0.1	0	0
B-7533	1	2.6 ± 0.1	15.9 ± 0.3	0.5 ± 0.1	21.6 ± 0.4	5.3 ± 0.1
	3	4.3 ± 0.1	19.1 ± 0.4	1.7 ± 0.1	45.8 ± 0.9	40.9 ± 0.8
B-4054	1	n.d.	0.6 ± 0.1	52.3 ± 1.0	8.7 ± 0.2	7.0 ± 0.1
	3	11.3 ± 0.2	5.6 ± 0.1	0	32.8 ± 0.6	33.6 ± 0.6
Control 1 (Hemopropiovit)	1	5.7 ± 0.1	45.3 ± 0.9	1.5 ± 0.1	95.5 ± 1.8	278.3 ± 5.3
	3	13.7 ± 0.3	26.1 ± 0.5	0	25.0 ± 0.5	0.6 ± 0.1
Control 2 (Biform)	1	0	0	1.1 ± 0.1	15.3 ± 0.3	28.1 ± 0.5
	3	0.5 ± 0.1	4.4 ± 0.1	0	10.3 ± 0.2	0

n.d. – not detected; 0 – detected but in a minimum threshold amount; time points: 1 – exponential phase; 3 – death phase

strains differed not only in the range of metabolites but also in the levels of their accumulation between the time points. For some strains, maximum acid concentrations were recorded during the exponential phase, while others showed maximum values during the death phase.

In comparison with the exponential phase, the death phase (time point 3) showed the most complete and stable metabolic profile, reflecting the strain's total metabolic activity throughout the entire growth cycle. By this point, the culture had undergone the main stages of adaptation to the environmental conditions accompanied by the accumulation or conversion of metabolites. For this reason, further metabolic profiling was conducted at time point 3, the phase at which the strains most fully manifest their metabolic characteristics.

Tables 9–10 present the contents of amino acids in the probiotic strains.

Our analysis revealed significant differences in the range and contents of amino acids synthesized by the strains. The highest total amino acid content was observed in Strain B-7509, with similar values recorded in B-10412. A slightly lower content was registered in B-7533, while no amino acids were detected in B-6080 and AC-1257. A similar situation was observed for the control samples.

Strain B-10412 synthesized a broad spectrum of amino acids, with high concentrations of individual compounds. The highest levels were recorded for tryptophan, glutamic acid, glycine, and isoleucine. The

strain's diverse profile also included tyrosine, cystine, arginine, phenylalanine, leucine, and other amino acids.

Strain B-7509 had the highest concentration of proline, as well as high levels of glycine, glutamic acid, isoleucine, serine, histidine, phenylalanine, alanine, and lysine. Strain B-7533 had a similar profile, with the highest contents of proline, glutamic acid, glycine, serine, histidine, isoleucine, phenylalanine, and methionine.

AC-1779 showed a moderate level of amino acid accumulation, with proline, glutamic acid, and glycine as the most prevalent compounds. Lower concentrations were detected for alanine, phenylalanine, lysine, and isoleucine.

Strain B-6083 had a relatively balanced amino acid profile. The highest concentrations were observed for proline and glycine. Moderate values were recorded for glutamic acid, isoleucine, alanine, and serine. Other amino acids were present in low concentrations.

B-4054 exhibited a relatively moderate overall content of amino acids, with some compounds accumulated in higher concentrations than others. The highest values were observed for proline, glycine, and isoleucine. Also present were tryptophan, glutamic acid, valine, alanine, and phenylalanine.

The synthesis and accumulation of free amino acids is an important metabolic characteristic of probiotic microorganisms. These compounds result from the proteolytic activity of bacteria and the hydrolysis of protein substrates during fermentation. Many lactic acid bacteria

Table 9 Contents of amino acids in the strains B-6083, B-6080, B-10412, AC-1779, and AC-1257

Amino acid	Amino acid content in samples, mg/g				
	B-6083	B-6080	B-10412	AC-1779	AC-1257
Tryptophan	1.7 ± 0.1	n.d.	32.9 ± 0.6	25.5 ± 0.5	n.d.
Glutamic	5.2 ± 0.1	n.d.	18.2 ± 0.3	11.1 ± 0.2	n.d.
Serine	3.0 ± 0.1	n.d.	1.5 ± 0.1	0.7 ± 0.1	n.d.
Glycine	12.1 ± 0.2	n.d.	13.2 ± 0.2	9.3 ± 0.2	n.d.
Histidine	1.6 ± 0.1	n.d.	4.4 ± 0.1	1.0 ± 0.1	n.d.
Glutamine	1.4 ± 0.1	n.d.	0.7 ± 0.1	1.7 ± 0.1	n.d.
Arginine	0.4 ± 0.1	n.d.	3.0 ± 0.1	0.8 ± 0.1	n.d.
Lysine	1.4 ± 0.1	n.d.	2.6 ± 0.1	3.1 ± 0.1	n.d.
Threonine	0.6 ± 0.1	n.d.	3.8 ± 0.1	0.9 ± 0.1	n.d.
Alanine	3.5 ± 0.1	n.d.	28.6 ± 0.5	1.8 ± 0.1	n.d.
Proline	13.2 ± 0.2	n.d.	5.0 ± 0.1	20.1 ± 0.4	n.d.
Asparagine	0.1 ± 0.1	n.d.	2.5 ± 0.1	0.5 ± 0.1	n.d.
Tyrosine	0.2 ± 0.1	n.d.	1.7 ± 0.1	0.8 ± 0.1	n.d.
Methionine	1.3 ± 0.1	n.d.	1.9 ± 0.1	1.6 ± 0.1	n.d.
Valine	3.1 ± 0.1	n.d.	0.9 ± 0.1	1.4 ± 0.1	n.d.
Cysteine	1.1 ± 0.1	n.d.	1.1 ± 0.1	0.7 ± 0.1	n.d.
Cystine	0.2 ± 0.1	n.d.	2.3 ± 0.1	0.4 ± 0.1	n.d.
Leucine	2.2 ± 0.1	n.d.	5.6 ± 0.1	0.5 ± 0.1	n.d.
Isoleucine	4.0 ± 0.1	n.d.	8.2 ± 0.2	1.8 ± 0.1	n.d.
Phenylalanine	2.3 ± 0.1	n.d.	3.9 ± 0.1	3.2 ± 0.1	n.d.
Total:	58.3	—	142.1	86.9	—

n.d. – not detected; “0” – detected but in a minimum threshold amount

Table 10 Contents of amino acids in the strains B-7509, B-7533, and B-4054, as well as Controls 1 and 2

Amino acid	Amino acid content in samples, mg/g				
	B-7509	B-7533	B-4054	Control 1	Control 2
Tryptophan	7.5 ± 0.1	0.9 ± 0.1	4.9 ± 0.1	n.d.	n.d.
Glutamic	17.4 ± 0.3	20.3 ± 0.4	4.0 ± 0.1	n.d.	n.d.
Serine	6.0 ± 0.1	6.2 ± 0.1	1.3 ± 0.1	n.d.	n.d.
Glycine	20.3 ± 0.4	15.3 ± 0.3	10.8 ± 0.2	n.d.	n.d.
Histidine	6.8 ± 0.1	6.8 ± 0.1	0.9 ± 0.1	n.d.	n.d.
Glutamine	4.3 ± 0.1	4.1 ± 0.1	1.6 ± 0.1	n.d.	n.d.
Arginine	2.7 ± 0.1	2.4 ± 0.1	0	n.d.	n.d.
Lysine	5.4 ± 0.1	4.7 ± 0.1	1.0 ± 0.1	n.d.	n.d.
Threonine	3.4 ± 0.1	3.4 ± 0.1	2.0 ± 0.1	n.d.	n.d.
Alanine	6.6 ± 0.1	4.5 ± 0.1	3.5 ± 0.1	n.d.	n.d.
Proline	32.0 ± 0.6	25.0 ± 0.5	15.6 ± 0.2	n.d.	n.d.
Asparagine	0.2 ± 0.1	1.3 ± 0.1	0.2 ± 0.1	n.d.	n.d.
Tyrosine	0.4 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	n.d.	n.d.
Methionine	3.7 ± 0.1	3.3 ± 0.1	0.7 ± 0.1	n.d.	n.d.
Valine	6.3 ± 0.1	4.5 ± 0.1	3.4 ± 0.1	n.d.	n.d.
Cysteine	3.3 ± 0.1	2.9 ± 0.1	0.9 ± 0.1	n.d.	n.d.
Cystine	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	n.d.	n.d.
Leucine	5.1 ± 0.1	3.6 ± 0.1	1.9 ± 0.1	n.d.	n.d.
Isoleucine	7.9 ± 0.1	5.8 ± 0.1	4.7 ± 0.1	n.d.	n.d.
Phenylalanine	5.7 ± 0.1	5.2 ± 0.1	2.1 ± 0.1	n.d.	n.d.
Total:	145.2	120.7	60	–	–

n.d. – not detected; “0” – detected but in a minimum threshold amount

exhibit high proteolytic activity, which breaks down proteins into peptides and free amino acids, which are involved in subsequent microbial metabolism. Ter *et al.* [92] noted that lactic acid bacteria are capable of actively hydrolyzing dietary proteins and forming a wide range of free amino acids, including proline, glycine, glutamic acid, leucine, and isoleucine. This has a significant effect on the functional properties of fermented products.

In a study of amino acid metabolism in fermented milk, Terzioğlu *et al.* [93] demonstrated that the probiotic cultures of *Lactobacillus* and *Bifidobacterium* contribute to the accumulation of alanine, glycine, proline, and glutamic acid, with their contents dependent on the composition of the microbial culture and the fermentation substrate.

Overall, our results indicate pronounced strain-specificity in the amino acid profiles of the cultures. The strains differed in the intensity of protein metabolism, which determined the formation of specific amino acid profiles. These metabolic characteristics can be used among the criteria for selecting promising cultures for further research and for developing functional products with increased amino acid contents.

Biocompatibility of the strains. Table 11 provides data on the biocompatibility of the probiotic strains with each other.

Biocompatibility analysis revealed that most interactions between the strains were neutral or stimulatory, with some strains exhibiting sensitivity to the metabolites of other microorganisms. For example, B-6083 biomass growth was inhibited by all metabolites except for AC-1779. Strain B-10412 was inhibited by AC-1779 and AC-1257, with other interactions being neutral.

Strains AC-1257, B-7509, and B-7533 exhibited mostly stimulatory effects, reflecting their high potential for co-cultivation. Notably, B-4054 was stimulated by metabolites from several strains (B-6083, AC-1779, AC-1257, and B-7509), while the remaining metabolites were neutral.

Thus, our results demonstrate that strain combinations with mostly stimulatory or neutral interactions can be used to form consortia with minimal conflicts between microorganisms. The most promising combinations are between AC-1257, B-7509, B-10412, and B-4054. Their metabolites promote each other's growth or have no negative impact on each other, ensuring the stability and efficiency of co-cultivation.

Table 11 Mutual biocompatibility of microbial metabolites

Microbial metabolites in culture broth	Strain biomass						
	B-6083	B-10412	AC-1779	AC-1257	B-7509	B-7533	B-4054
B-6083	–	–	++	++	+	+	++
B-10412	–	–	++	++	+	+	+
AC-1779	+	–	–	+	++	++	++
AC-1257	–	–	+	–	++	++	++
B-7509	–	+	++	++	–	+	++
B-7533	–	+	++	++	+	–	+
B-4054	–	+	+	+	++	++	–

“+” – biocompatibility; “++” – growth stimulation; “–” – growth inhibition

CONCLUSION

Our comprehensive assessment of the probiotic strains revealed those that are sensitive to clinically significant antibiotics, resistant to gastrointestinal stress factors, and capable of high antagonistic activity against opportunistic microflora. Based on their combined properties, the most promising strains are *Pediococcus acidilactici* B-7509 and B-7533, *Bifidobacterium longum* AC-1257, *Weissella thailandensis* B-10412, and *Enterococcus faecium* B-4054.

Metabolic analysis demonstrated their ability to synthesize bioactive compounds, including B vitamins, organic acids, and amino acids. The highest amino acid content was observed in *P. acidilactici* B-7509 (145.2 mg/g) and *W. thailandensis* B-10412 (142.1 mg/g).

Biocompatibility analysis revealed mostly neutral or stimulatory interactions between the strains. The most promising combinations for probiotic consortia are between *B. longum* AC-1257, *P. acidilactici* B-7509 and B-7533, *W. thailandensis* B-10412, and *E. faecium* B-4054.

According to our results, these strains have high potential as components of dietary supplements and

functional foods [94]. However, their potential use in starter cultures requires additional research to assess their safety and technological properties in accordance with regulatory requirements.

The strains' antagonistic activity and resistance to gastrointestinal factors indicate their potential effectiveness when administered orally. According to current research, probiotic metabolites can have a positive effect on lipid metabolism (reducing cholesterol and triglyceride levels) [95], carbohydrate metabolism [96], and the gut microbiota [40]. Therefore, these cultures need to be further studied as part of probiotic consortia. The next stage would be to evaluate their functional activity *in vivo* using animal models.

CONTRIBUTION

The authors were equally involved in writing this manuscript and bear equal responsibility for plagiarism.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this publication.

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