PHARMACIA

Pharmacia 73: e189800
DOI 10.3897/pharmacia.73.e189800

Research Article

Antimicrobial properties of postbiotics of different LAB against multidrug resistance bacteria

Flora Tkhruni[1], Svetla Danova[2], Gayane Melik-Andreasyan[3], Naira Aleksanyan[3], Arevik Israyelyan[1]

1 *Probiotic Biotechnology Laboratory, Scientific and Production Center "Armbiotechnology" NSPO, National Academy of Sciences, Yerevan, Armenia*
2 *Bulgarian Academy of Sciences, Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria*
3 *National Center for Disease Control and Prevention, SNCO, Ministry of Health, Yerevan, Armenia*

Corresponding author: Arevik Israyelyan (arevik_israelyan@mail.ru)

Received 25 February 2026 ♦ **Accepted** 28 July 2026 ♦ **Published** 24 August 2026

Citation: Tkhruni F, Danova S, Melik-Andreasyan G, Aleksanyan N, Israyelyan A (2026) Antimicrobial properties of postbiotics of different LAB against multidrug resistance bacteria. Pharmacia 73: e189800. https://doi.org/10.3897/pharmacia.73.e189800

Abstract

It was shown the comparative antimicrobial activity of postbiotics (bacteriocins and exopolysaccharides) isolated from lactic acid bacteria (LAB) strains of genus Enterococcus and Lactobacillus against three broad panelsof human pathogens. The antimicrobial activity was assessed in a concentration-dependent manner and compared with that of conventional antibiotics. Selected and purified bacteriocins from strains of *L. rhamnosus* 2012 and *E. faecium* KE5, for which an MIC of 5 mg/mL was established against *S. typhimurium* G-38 via the serial dilution method, exhibited the strongest antimicrobial activity across a broad panel of pathogens when tested at this concentration using agar diffusion assays. Postbiotics displayed strain-specific inhibitory effects, highlighting their narrower antimicrobial spectrum relative to antibiotics. It was shown that conventional antibiotics demonstrated broad-spectrum efficacy against susceptible strains but known failures against multidrug-resistant (MDR) isolates. Differences in antimicrobial effects between postbiotics may depend on their interaction with the cell membrane of pathogens. These findings suggest that LAB-derived postbiotics could serve as complementary antimicrobial agents (with antibiotics or another bioactive substance), which may have applications in nutritional and preventive frameworks.

Keywords

Antibiotic, bacteriocin, exopolysaccharide, LAB, multidrug resistance bacteria

Introduction

For decades, antibiotics have been the cornerstone of antimicrobial therapy, effectively targeting a broad range of pathogenic bacteria. However, their widespread and often indiscriminate use has contributed to the emergence of multidrug-resistant strains, disruption of the host microbiome, and increased incidence of antibiotic-associated complications. The problem is especially pronounced among Gram-negative bacteria, which the report identifies as posing "the greatest threat," particularly in resource-limited settings where surveillance and treatment options are constrained (Seid et al. 2025; WHO 2025). These worrying global patterns underscore the need for comprehensive surveillance and analysis across diverse bacterial taxa. In clinical practice, the main culprits responsible for serious infections—urinary,

bloodstream, respiratory, and nosocomial—frequently belong to three broad groups: Gram-negative Enterobacteriaceae**,** Gram-negative non-fermenters**,** and Gram-positive cocci**.** Each group has unique biology, ecology, and mechanisms of resistance, which influence how susceptibility evolves over time and across regions (Horice 2025; Wang et al. 2025).

These challenges have prompted growing interest in alternative or complementary antimicrobial approaches that offer efficacy with fewer unintended consequences.

In parallel, growing attention is being directed toward postbiotics (bacteriocins and exopolysaccharides) as a promising alternative or adjunct to traditional antibiotics. Postbiotics, which include inactivated microbial cells and their bioactive metabolites, exhibit broad antimicrobial activity against pathogenic bacteria (Moradi 2020; Rahman et al. 2025). They can suppress pathogen growth, disrupt biofilm formation, and attenuate virulence without exerting strong selective pressure for resistance. Furthermore, postbiotics enhance host immune responses and improve gut barrier integrity, contributing to infection prevention. Their stability, safety, and reproducibility make them suitable for therapeutic and preventive applications. Incorporating postbiotics into antimicrobial strategies may help reduce antibiotic dependence and support global efforts to combat antimicrobial resistance. The obtained results demonstrate that the isolated and purified postbiotics are capable of inhibiting the growth of pathogenic strains responsible for a range of human diseases, including pneumonia, and may serve as a basis for the development of next-generation antibiotics (Tkhruni et al. 2023; Israyelyan et al. 2024).

Among these emerging strategies, postbiotics- have gained significant attention. Unlike live probiotics, postbiotics retain functional bioactivity without the risks associated with administering live bacteria, such as infection in immunocompromised individuals or instability during processing and storage. Their antimicrobial potential arises from diverse mechanisms, including the production of organic acids, bacteriocins, peptides, and other bioactive metabolites capable of inhibiting pathogenic microorganisms. LAB produces a variety of antimicrobial metabolites, including organic acids, hydrogen peroxide (H2O2), carbon dioxide, bacteriocins, and exopolysaccharides, collectively referred to as postbiotics (Cuevas-González et al. 2020). International Scientific Association for Probiotics and Prebiotics (ISAPP) defines postbiotics as "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Salminen et al. 2021). Investigated LAB strains such as *Lactobacillus* and *Bifidobacterium* release these compounds into their cell-free supernatant (CFS) during growth (Moradi 2020), thereby contributing to pathogen inhibition (Koohestani et al. 2018).

Bacteriocins are ribosomally synthesized peptides or proteins classified as "Generally Regarded As Safe" (GRAS) (Zapaśnik et al. 2022). They exhibit either broad- or narrow-spectrum bactericidal and bacteriostatic activity against foodborne pathogens and spoilage bacteria (Thuy et al. 2024). Due to their low toxicity, high thermal and pH stability, salt tolerance, lack of resistance development and immunogenicity, and susceptibility to digestive proteases, these compounds are ideal candidates for safe food preservatives (Manna and Mondal 2023; Shafique et al. 2023; Daba et al. 2024). Derived from various LAB species and strains, bacteriocins show significant antimicrobial activity against both Gram-positive and Gram-negative bacteria and remain stable across varying environmental conditions (Kurnianto et al. 2021; Peng 2023).

Exopolysaccharides (EPSs) are another critical group of LAB-derived postbiotics. These high-molecular-weight carbohydrate polymers can either remain loosely attached to the bacterial cell surface or be secreted into the surrounding environment (Li et al. 2017; Angelin and Kavitha 2020). EPSs have garnered growing attention for their multifaceted roles in host-microbe interactions, including immune modulation, antitumor activity, antioxidant enhancement, and cholesterol-lowering effects. Moreover, certain EPSs have demonstrated selective prebiotic effects by promoting the growth of beneficial gut microbiota such as bifidobacteria and lactobacilli (Mokoena 2017; Nehal et al. 2019; Jurášková et al. 2022).

It was shown that strains of Enterococcus with probiotic properties—are capable of producing both bacteriocins and EPSs, each with distinct antimicrobial profiles. Notably, not all EPSs produced under identical conditions display antimicrobial activity. Our results demonstrated that strains of Enterococcus, especially those isolated from fermented donkey milk (matsoni), yielded the highest quantities of EPSs. Purified EPSs from strains used in this work exhibited antimicrobial activity against a broad range of pathogenic bacteria, including *Salmonella* spp., *Escherichia coli*, *Proteus mirabilis*, *Pasteurella* spp., *Clostridium* spp., *Streptococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae*, indicating both probiotic and immunomodulatory potential (Tkhruni et al. 2023; Israyelyan et al. 2024). Despite increasing interest in LAB-derived postbiotics, most existing literature focuses primarily on the inhibitory effects of bacteriocins against specific pathogens, like *E. coli*, *S. typhimurium*, and *Staph. aureus* (Aghamohammad et al. 2025). Limited research has evaluated both Gram-positive and Gram-negative including MDR strains.

The aim of this study focuses on postbiotics (EPS and BCN) derived from 6 different selected probiotic LAB strains and comparative antimicrobial efficacy of antibiotics and LAB-derived postbiotics against abroad range of human pathogens, including groups of Gram-negative Enterobacteriaceae, non-fermenting bacteria, and Gram-positive cocci.

Materials and methods

Microbial strains and culture conditions

In the study were used endemic lactic acid bacteria (**LAB**) strains isolated from traditional fermented milk: *Enterococcus faecium* KE5, *Enterococcus faecium* KE13,

Enterococcus faecium KE14, *Enterococcus durans* KE6, *Lactobacillus rhamnosus* 2012, and *Lactobacillus helveticus* KG5 (Israyelyan 2018). These strains are preserved at the Microbial Depository Center (**MDC**) of SPC "Armbiotechnology" NAS RA and are registered in the World Data Centre for Microorganisms (**WDCM** database, (www.armbiotech.am; www.wfcc.info) database. Stock cultures were maintained at −20 °C in MRS (Himedia) broth supplemented with 40% (v/v) glycerol.

Reference strains were obtained from internationally recognized culture collections including the American Type Culture Collection (**ATCC**) (https://www.atcc.org/), the National Collection of Type Cultures of the United Kingdom (**NCTC**) (https://www.culturecollections.org.uk/collections/nctc), the Microbiological Quality Control Laboratory in Darmstadt, Germany (**MQCL**), and the type cultures provided by the United Kingdom National External Quality Assessment Service (**UK NEQAS**) (https://www.ukneqas.org.uk/). The test panel included representatives of:

- Enterobacteriaceae: *Escherichia coli* (ATCC 25922, MQCL 6578), *Proteus mirabilis* (UKNEQAS 4571/5275, 5243/7400), *Klebsiella pneumoniae* (ATCC 13883, 700603).
- Non-fermenters: *Pseudomonas aeruginosa* (ATCC 27853, UKNEQAS 5290/4576), *Acinetobacter baumannii* (UKNEQAS 7601/5307, ATCC 13301, OXA-48 positive).
- Gram-positive cocci: *Staphylococcus aureus* (ATCC 29213, NCTC 12493, MRSA), *Streptococcus pneumoniae* (ATCC 49619, UK NEQAS 4907), *Enterococcus faecalis* (ATCC 29212, NCTC 13379, HLAR).

Used reference strains were selected to represent both antimicrobial-susceptible and antimicrobial-resistant phenotypes.

Media preparation for postbiotic production

For the production of postbiotics (bacteriocins and exopolysaccharides), specific enriched media were used:

- Curd Whey Medium: specialized medium was prepared based on 100 mL of curd whey supplemented with the following salts (%, w/v): $(NH_4)_2SO_4$ (0.8), KH_2PO_4 (0.1), $MgSO_4$ (0.2), yeast extract (0.3), peptone (0.3), $MnSO_4$ (0.05), and $CH_3COONa \times 3H_2O$ (0.2), pH 5.5–6.5, following the formulation according (Tkhruni et al. 2015).

Cultivation of LAB

Each LAB strain was separately cultivated sterile curd whey medium using a 10% (v/v) inoculum of overnight culture. Fermentation was carried out under anaerobic conditions at 30 °C, 48 h. for isolated exopolysaccharides and 37 °C, 48 h for isolated bacteriocin.

Production of postbiotic

Following incubation, cultures were centrifuged at 6000 × g for 20 min at 4 °C to obtain a cell-free culture (CFC) supernatant. The supernatant was concentrated five-fold (CFC × 5) using a rotary evaporator at 50 °C under reduced pressure (0.01 MPa), resulting in a dry matter content of approximately 8–12%. Subsequently, biological active substances were isolated and purified.

Isolation and purification of postbiotics

Bacteriocins (BCN)

The concentrated broth was subjected to gel filtration on a Sephadex G-25 column (1.5 × 57 cm, 100 mL resin volume; Sigma-Aldrich) for the isolation of protein-like substances (bacteriocins). The column was washed with sterile distilled water. Fractions exhibiting antimicrobial activity were pooled and vacuum-dried at 50–55 °C (0.01 MPa). A total of 30–50 fractions (5 mL each) were collected and analyzed by UV spectrophotometry (230, 260, and 320 nm) using a UV/VIS spectrophotometer (2800, Hitachi). Fractions showing high antimicrobial activity against test culture were combined for further study. Selected fractions of BCN were maintained at +4 °C.

Exopolysaccharides (EPS)

For EPS production, LAB were cultivated in curd whey medium. The precipitated proteins were removed by centrifugation (6000 × g for 20 min at 4 °C) and 96% ethanol was added to the supernatant in a 1:3 ratio. The solution was kept at 4 °C for another 24 h to precipitate EPS. The resulting EPS pellet was dried in a thermostat at 50 °C until a constant weight was achieved (Sørensen et al. 2022). The concentrated EPS solution (5 mL) was further purified by gel filtration chromatography on a Sephadex G-50 column (1.5 × 57 cm, 100 mL resin volume; Sigma-Aldrich). The column was washed with sterile distilled water. Fractions (30–50 aliquots of 5 mL each) were collected and analyzed by UV spectrophotometry (200–600 nm) using a UV/VIS spectrophotometer (2800, Hitachi). Fractions demonstrating the highest antimicrobial activity against test culture were combined for further study. Selected fractions of EPS were maintained at +4 °C.

Characterization of postbiotics

The molecular weight (Mw) of purified EPS and BCN was determined by Fast Protein Liquid Chromatography (FPLC) using TSK G-5000 PWXL and G-3000 PWXL columns. Detection was performed using a refractive index detector (Waters 2414). Mw values for EPS were calculated based on calibration curves generated using dextran standards (Sigma-Aldrich), while bacteriocin molecular weights were estimated using protein molecular weight markers (Suppl. material 1) (Salminen et al. 2021).

Monosaccharide composition analysis high-performance liquid chromatography (HPLC)

Fractions with bactericidal activity, obtained after gel filtration method, were purified by HPLC method with application of various systems (Semi-preparative "Avex ODS" C_{18} column (8 by 250 mm, Waters and Shimadzu, Japan); Shimadzu LC-20 analytical C_{18} column (4.6 by 250 mm, Symmetry, USA, with a detector Diode array SPD-20a, auto-sampler). The eluent consisted of bi-distilled water, trifluoroacetic acid and acetonitrile (HPLC "SIGMA", USA)). Sample injection volume was 100 µl. Elution monitored at different wave lengths ranged from190 nm to 400 nm. Detection was performed at 210, 254, 280 nm wave lengths. Fractions eluted from the column were freeze- dried, dissolved in 150 µl of bi-distilled water and tested for antibacterial activity. Fractions, showing maximal antimicrobial activity, have been selected. HPLC analysis confirmed that the isolated EPS fractions are heteropolysaccharides composed primarily of glucose and galactose (Salminen et al. 2021).

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the indicator strains was determined according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Results were interpreted as susceptible (S), intermediate (I), or resistant (R) according to EUCAST breakpoints (EUCAST, https://www.eucast.org). Each assay was performed in three independent replicates**,** and all measurements included negative controls (sterile medium) and positive controls (reference antibiotics) to verify the responsiveness of reference strains. Antibiotic panels included: Enterobacteriaceae: β-lactams, cephalosporins (I–IV generation), monobactams, fluoroquinolones, aminoglycosides, carbapenems, sulfonamides, chloramphenicol, fosfomycin, nitrofurans, nitroxoline. Non-fermenters: Piperacillin-tazobactam, ceftazidime, ceftazidime-avibactam, cefepime, aztreonam, fluoroquinolones, aminoglycosides, carbapenems, trimethoprim-sulfamethoxazole. Gram-positive cocci: Penicillins, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, glycopeptides, oxazolidinones, tetracyclines, sulfonamides, macrolides, nitrofurans, fusidic acid.

Results were interpreted as susceptible (S), intermediate (I), or resistant (R) according to EUCAST breakpoints. The work was done by bacteriologist in National Center for Disease Control and Prevention, SNCO, Ministry of Health, Republic of Armenia. This method was specifically selected at the National Center for Disease Control and Prevention (SNCO, Ministry of Health, RA) to facilitate a direct biological comparison between the postbiotic preparations and standard antibiotic discs used in clinical practice.

Detection of resistance mechanisms

Resistance mechanisms including extended-spectrum β-lactamases (ESBLs), carbapenemases, methicillin resistance (MRSA), and high-level aminoglycoside resistance (HLAR) were detected using standard phenotypic methods, including the double-disk synergy test, modified Hodge test, and Carba NP test. The presence of carbapenemase genes (KPC, NDM, OXA) was confirmed by polymerase chain reaction (PCR).

Determination of antimicrobial activity of postbiotics

Antimicrobial activity of purified BCN and EPS fractions was determined using the agar diffusion (drop) method following CLSI recommendations (M02-A13 2023). Indicator strains were cultured overnight and adjusted to 0.5 McFarland standard (approximately 1×10^8 CFU/mL). The bacterial suspension was uniformly spread onto Mueller–Hinton agar plates and 20 µL of each of the samples was applied onto the agar surface. Plates were incubated at 37 °C for 18–24 h, and antimicrobial activity was evaluated by measuring the diameter of inhibition zones (mm). Activity was expressed as arbitrary units per milliliter (AU/mL) according to Parente et al. (1995). One arbitrary unit was defined as the reciprocal of the highest two-fold dilution of the sample that produced a detectable inhibition zone against the indicator microorganism. Sterile culture medium processed under identical conditions served as the negative control.

Data presentation and statistical analysis

All experimental procedures were performed in three independent replicates to ensure data reproducibility. Quantitative results, including the diameters of growth inhibition zones (mm) and the calculated activity parameters (AU/mL), are expressed as the mean ± standard deviation (SD). Prior to performing comparative tests, the normality of the data distribution was assessed. For the evaluation of antimicrobial susceptibility, indicator strains were categorized as Susceptible (S), Intermediate (I), or Resistant (R) according to EUCAST breakpoints. Statistical processing was carried out using SPSS version 28 or R software. In all analyses, a p-value of less than 0.05 was considered to indicate statistical significance. Results were visualized through structured tables and high-resolution figures to facilitate the comparative assessment of postbiotic activity against various reference pathogens.

Results

The Minimum Inhibitory Concentration (MIC) was determined during the initial screening stage against the model organism *Salmonella typhimurium* G-38 using the serial dilution method, yielding an effective concentration of 5 mg/mL. This concentration was subsequently employed as the standardized dose for further testing against a broader panel of clinical and reference strains using the agar diffusion (drop) method to facilitate comparative analysis . This stepwise approach allowed for the preliminary identification of effective concentrations and facilitated subsequent comparative analysis of

postbiotic preparations (EPS and BCN) and antibiotics across diverse bacterial targets.

The antimicrobial activity of EPS was assessed across a concentration range of 0.1–200 mg/mL. It was shown that all EPS preparations produced nearly complete growth inhibition (~100%) at 100 mg/mL, confirming strong activity at high concentrations except *E. durans* KE6, growth inhibition (~50%) at 200 mg/mL. The Fig. 1A shows data for only 4 EPS, because EPS *E. faecium* KE13 and *E. faecium* KE14 showed the same effect as *E. faecium* KE5. However, marked differences in concentration-dependent efficacy were observed among strains. EPS from *E. faecium* KE5 demonstrated a more gradual decline in inhibitory activity, retaining measurable antimicrobial effects at intermediate concentrations (50–1 mg/mL), suggesting greater potency and possible lower effective inhibitory thresholds. In contrast, EPS derived from *L. rhamnosus* 2012 exhibited a steep reduction in activity below 100 mg/mL, with inhibition approaching negligible levels at ≤ 10 mg/mL. EPS from *L. helveticus* KG5 and *E. durans* KE6 also showed pronounced reductions in activity at lower concentrations, indicating weaker dose sustainability. It can be assumed that, at 50–1 mg/mL, concentrations, EPS is associated with the presence of specific targets, where maximum binding to high-affinity targets occurs which have the same significant with some antibiotics. These findings highlight strain-dependent variability in EPS bioactivity and suggest that structural or compositional differences among EPS may contribute to the observed differences in antimicrobial efficacy.

The antimicrobial activity of BCN was evaluated over a concentration range of 50–1 mg/mL. All BCN preparations exhibited strong inhibitory activity at the highest concentrations tested (10–50 mg/mL), achieving approximately 95–100% growth inhibition (Fig. 1B). However, distinct strain-dependent differences became evident as concentrations decreased. BCN derived from *E. faecium* KE5 maintained high inhibitory activity down to 10 mg/mL and retained substantial activity even at 5 mg/mL, indicating superior potency compared to the other investigated bacteriocins. In contrast, BCN from *L. helveticus* KG5 and *L. durans* KE6 demonstrated a pronounced reduction in antimicrobial activity at 10 mg/mL and dropped sharply at 5 mg/mL. BCN2 from *L. rhamnosus* 2012 showed intermediate stability, maintaining moderate inhibition at 10 mg/mL but exhibiting a significant decline at lower concentrations. Overall, bacteriocin from *E. faecium* KE5 displayed the most sustained antimicrobial effect across decreasing concentrations. The differences in effectiveness depended on the strains used, the molecular weight of the postbiotics, and their specific interaction mechanisms with the pathogen cell membranes. Previously, it was shown that *L. rhamnosus* 2012 has 2 bacteriocin (BCN1 – 1.42×10^3 Da, BCN2 – 6.02×10^2 Da) which have different molecular weight and antimicrobial activity against different pathogens (Tkhruni et al. 2020; Grigoryan et al. 2025).

The effectiveness of obtained EPS and BCN was investigated using antibiotics. The antimicrobial susceptibility profile of six Enterobacteriaceae strains, including *E. coli*, *P. mirabilis* and *Klebsiella pneumoniae*, against conventional antibiotics and postbiotics is summarized in Fig. 2.

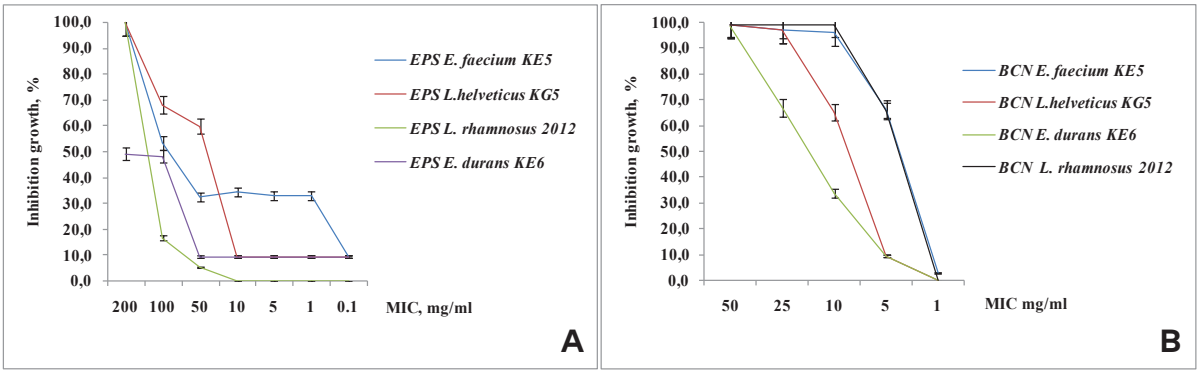
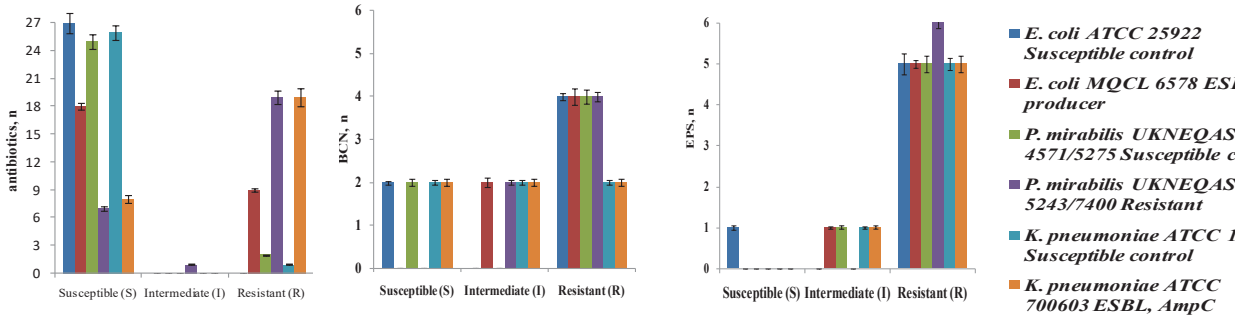


Figure 1. Comparative antimicrobial activity of postbiotics (EPS and BCN), test culture (*Salmonella typhimurium* G-38).



Legends: Antibiotic-standard dose, BCN-5 mg/mL, EPS-5 mg/mL

Figure 2. Antimicrobial susceptibility profile of Enterobacteriaceae isolates against antibiotics and postbiotics.

The antimicrobial susceptibility profile of Enterobacteriaceae isolates (n = 27) demonstrated variable responses to conventional antibiotics and postbiotic preparations (Fig. 2). Overall, the majority of isolates were classified as susceptible to the tested antibiotics, with minimal representation in the intermediate category. However, resistant phenotypes were evident, particularly among extended-spectrum β-lactamase (ESBL)–producing strains, including *Escherichia coli* MQCL 6578 and *Klebsiella pneumoniae* ATCC 700603. Susceptible control strains such as *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883 showed high levels of susceptibility, as expected. In contrast, the postbiotic preparations BCN (n = 6) and EPS (n = 6) demonstrated reduced antimicrobial activity. For BCN, a moderate distribution between susceptible and intermediate categories was observed; however, a considerable proportion of isolates were resistant. EPS exhibited the lowest overall efficacy, with the majority of isolates categorized as resistant and only minimal susceptibility detected.

Collectively, these findings indicate that while conventional antibiotics retain activity against a substantial proportion of isolates, resistance remains prominent among ESBL- and AmpC-producing strains. The tested postbiotics displayed comparatively limited inhibitory effects against the evaluated Enterobacteriaceae isolates.

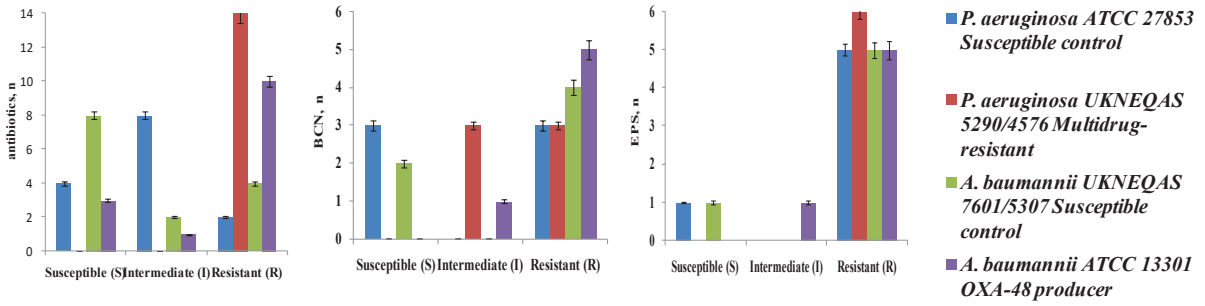
The susceptibility of Gram-Negative Non-Fermenting Bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains to conventional antibiotics and postbiotics is summarized in Fig. 3.

The antimicrobial susceptibility profile of Gram-negative non-fermenting bacteria demonstrated marked variability between antibiotics and postbiotic preparations (Fig. 3). Among antibiotic-tested isolates, susceptible responses were predominantly observed in the control strains, including *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* UKNEQAS 7601/5307. In contrast, the multidrug-resistant strain *Pseudomonas aeruginosa* UKNEQAS 5290/4576 and the OXA-48–producing *Acinetobacter baumannii* ATCC 13301 exhibited high levels of resistance, with the majority of tested antibiotics categorized as resistant and only limited intermediate susceptibility.

Similarly, BCN demonstrated limited inhibitory effects, with resistance predominating across strains, particularly among resistant phenotypes. Some susceptible responses were observed in control strains; however, resistant isolates maintained high resistance levels.

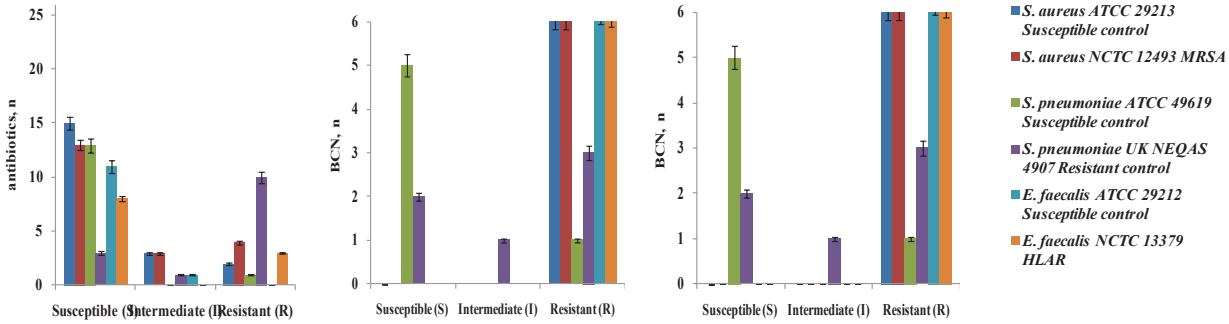
Overall, the findings indicate that Gram-negative non-fermenting bacteria, especially multidrug-resistant and carbapenemase-producing strains, exhibit substantial resistance not only to conventional antibiotics but also to the tested postbiotic preparations.

The susceptibility of Gram-positive cocci, including Staphylococcus aureus, Streptococcus pneumoniae, and *Enterococcus faecalis*, to conventional antibiotics and postbiotics is summarized in Fig. 4.



Legends: Antibiotic-standard dose, BCN-5 mg/mL, EPS-5 mg/mL

Figure 3. Antimicrobial susceptibility of gram-negative non-fermenting bacteria against antibiotics and postbiotics.



Legends: Antibiotic-standard dose, BCN-5 mg/mL, EPS-5 mg/mL

Figure 4. Antimicrobial susceptibility of gram-positive cocci against antibiotics and postbiotics.

The antimicrobial susceptibility profile of Gram-positive cocci revealed clear differences between control and resistant strains when exposed to antibiotics and postbiotic preparations (Fig. 4). High susceptibility rates were observed among control strains, including *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, and *Enterococcus faecalis* ATCC 29212. In contrast, resistant phenotypes—*Staphylococcus aureus* NCTC 12493, *Streptococcus pneumoniae* UKNEQAS 4907, and *Enterococcus faecalis* NCTC 13379—demonstrated increased resistance to the tested antibiotics, with reduced susceptible and increased resistant classifications. Conventional antibiotics maintained activity against susceptible control strains, resistant Gram-positive cocci exhibited substantial resistance profiles. Both postbiotic preparations (BCN and EPS) demonstrated comparatively low efficacy against the evaluated isolates.

Initial screening against the test culture *Salmonella typhimurium* G-38 revealed marked strain-dependent variability in antimicrobial efficacy. While all BCN and EPS preparations exhibited inhibitory activity at high concentrations (10–50 mg/mL), *E. faecium* KE5 and *L. rhamnosus* 2012 demonstrated superior potency. *E. faecium* KE5 was distinguished by its 'dose sustainability,' maintaining significant inhibitory effects even at a lower concentration of 5 mg/mL, whereas other strains showed a sharp decline. Additionally, *L. rhamnosus* 2012 was selected due to its unique production of two distinct bacteriocins (BCN1 and BCN2), which broadens its inhibitory spectrum. Consequently, these two strains were prioritized for the final comparative evaluation against the broad panel of reference pathogens presented in Table 1.

Among Enterobacteriaceae susceptible control strains (*E. coli* ATCC 25922 and *P. mirabilis* UKNEQAS 4571/5275), high susceptibility rates (90–100%) were observed across all antibiotic classes. In contrast, the ESBL- and AmpC-producing *K. pneumoniae* ATCC 700603 demonstrated complete resistance to β-lactams (0%) and markedly reduced susceptibility to carbapenems (5%), while remaining fully susceptible to fluoroquinolones and aminoglycosides.

Non-fermenting pathogens displayed greater variability. *Pseudomonas aeruginosa* ATCC 27853 showed low susceptibility to β-lactams (10%) and fluoroquinolones (0%), moderate susceptibility to aminoglycosides (66.6%), and limited susceptibility to carbapenems (33.3%). Similarly, *Acinetobacter baumannii* exhibited resistance to β-lactams (0%) and partial susceptibility to fluoroquinolones (50%), while maintaining high susceptibility to aminoglycosides and carbapenems (100%). Among Gram-positive cocci, the susceptible control strain of *S. pneumoniae* ATCC 49619 demonstrated high susceptibility across antibiotic groups (71.5–100%). Conversely, the resistant control strain (*S. pneumoniae* UK NEQAS 4907) showed markedly reduced susceptibility to β-lactams (28.5%), complete resistance to fluoroquinolones and aminoglycosides (0%), and partial susceptibility to carbapenems (66.6%).

Notably, BCN preparations derived from *L. rhamnosus* 2012 and *E. faecium* KE5 exhibited consistent inhibitory activity (100%) across all tested pathogen strains, irrespective of resistance phenotype. In contrast to the variability observed among conventional antibiotic classes, BCN demonstrated uniform activity against both susceptible and resistant reference strains.

Discussion

The results of this study highlight a fundamental functional divergence between the two types of postbiotics isolated from endemic lactic acid bacteria (LAB). The bacteriocins (BCN) investigated—low-molecular-weight peptides ranging from 0.6 to 1.42 kDa—demonstrated significantly higher antimicrobial potency than the exopolysaccharides (EPS), which are high-molecular-weight polymers (10 to 44 kDa). It is important to acknowledge the limitations of diffusion-based assays, particularly for high-molecular-weight EPS (up to 44 kDa), where diffusion coefficients may impact the observed inhibition zones. This disparity likely reflects their distinct biochemical mechanisms: while BCNs typically exert direct bactericidal effects through membrane permeabilization and pore formation, the antimicrobial action of EPS is often indirect, potentially involving interference with bacterial adhesion or modulation of the microenvironment.

Table 1. Susceptibility profile of reference bacterial strains to selected BCN and major antibiotic classes (%).

Pathogens		Resistance phenotype of reference strains	BCN		Antibiotic classes			
			L. rhamnosus 2012	*E. faecium* KE5	Beta-lactams	Fluoroquinolones	Aminoglycosides	Carbapenems
Enterobacteriaceae	*E. coli* ATCC 25922	Susceptible control	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
	P. mirabilis UKNEQAS 4571/5275	Susceptible control	100 ± 0.0	100 ± 0.0	90 ± 1.5	100 ± 0.0	100 ± 0.0	100 ± 0.0
	K. pneumoniae ATCC 13883	Susceptible control	100 ± 0.0	100 ± 0.0	91 ± 1.5	100 ± 0.0	100 ± 0.0	100 ± 0.0
	K. pneumoniae ATCC 700603	ESBL, AmpC	100 ± 0.0	100 ± 0.0	0	100 ± 0.0	100 ± 0.0	5 ± 1.8
Gram-negative non-fermenters	*P. aeruginosa* ATCC 27853	Susceptible control	100 ± 0.0	100 ± 0.0	10 ± 1.7	0	66.6 ± 2.3	33.3 ± 2.5
	A. baumannii UKNEQAS 7601/5307	Susceptible control	100 ± 0.0	100 ± 0.0	0	50 ± 1.8	100 ± 0.0	100 ± 0.0
Gram-positive cocci	*S. pneumoniae* ATCC 49619	Susceptible control	100 ± 0.0	100 ± 0.0	71.5 ± 2.4	100 ± 0.0	100 ± 0.0	100 ± 0.0
	S. pneumoniae UK NEQAS 4907	Resistant control	100 ± 0.0	100 ± 0.0	28.5 ± 2.5	0	0	66.6 ± 2.3

Notably, *E. faecium* KE5 exhibited superior "dose sustainability," maintaining high inhibitory activity even at a concentration of 5 mg/mL, whereas other strains like *L. helveticus* KG5 and *E. durans* KE6 showed sharp declines at this threshold. This suggests that the BCN from KE5 may possess a higher affinity for specific targets on the pathogen cell membrane, a critical factor for therapeutic potential. The most significant scientific contribution of this study is the demonstration that LAB-derived BCNs retained consistent inhibitory activity (100%) against several resistant reference strains under the experimental conditions employed. As shown in our comparative analysis, conventional antibiotics suffered from a profound "erosion of susceptibility" against multidrug-resistant (MDR) phenotypes. For instance, the ESBL- and AmpC-producing *K. pneumoniae* ATCC 700603 demonstrated complete resistance to β-lactams (0%) and minimal susceptibility to carbapenems (5%). The greater antimicrobial potency observed for bacteriocins compared to EPS likely reflects fundamental differences in their biochemical nature and mechanisms of action. Bactericidal effects of bacteriocins are antimicrobial peptides that typically exert targeted bactericidal effects through membrane permeabilization, pore formation, or disruption of essential cellular processes. These proposed mechanisms are presented as hypotheses, as specific membrane permeability assays or SEM/TEM imaging were not conducted in this study. In contrast, exopolysaccharides are high-molecular-weight polymers whose antimicrobial effects are often indirect, potentially involving interference with adhesion, biofilm formation, or modulation of environmental conditions rather than direct membrane disruption.

Previous studies have demonstrated that the bacteriocin and exopolysaccharide produced by this strain exhibit antimicrobial activity against pneumonia-causing pathogens (Israyelyan et al. 2024). In stark contrast, BCN preparations from *L. rhamnosus* 2012 and *E. faecium* KE5 maintained 100% consistent inhibitory activity against these same MDR strains, regardless of whether the pathogens carried KPC, NDM, or OXA-type carbapenemases. This consistent efficacy suggests that these postbiotics may operate through pathways—potentially involving membrane-associated mechanisms—that appear to remain active despite the presence of enzymatic or efflux-based resistance mechanisms in the tested strains; however, the exact pathways remain to be experimentally confirmed. Specifically, the identification of two distinct bacteriocins (BCN1 and BCN2) in *L. rhamnosus* 2012—with molecular weights of 1.42 kDa and 0.60 kDa respectively—corroborates earlier work by Tkhruni et al. (2020), which suggested that multiple bioactive compounds contribute to the broad inhibitory spectrum of this BCN. Our data indicate the possibility of developing complex preparations this BCN with EPS with antimicrobial properties, or with different antimicrobial drugs, which can enhance the antimicrobial effect and reduce the likelihood of resistance and antibiotic concentration. The novelty of this study lies in the characterization of specific endemic strains (*E. faecium* KE5, *L. rhamnosus* 2012) that produce postbiotics exhibiting consistent inhibitory activity against resistant phenotypes under the experimental conditions employed. While conventional antibiotics showed limited effectiveness against resistant Gram-negative non-fermenters like *A. baumannii* (OXA-48 positive) and *P. aeruginosa* ATCC 27853 (0% susceptibility to fluoroquinolones), the tested BCNs remained fully effective (100%). These findings position endemic Armenian LAB strains as a valuable reservoir for novel antimicrobial agents capable of addressing the global crisis of antimicrobial resistance (AMR).

Other authors have demonstrated that in production of various probiotic drugs and functional food products, it is considered preferable to use strains with a good ability to synthesize bacteriocins. These inhibit pathogens directly; they are able to modulate the composition of the microbiota positively and stimulate the host immune system (Dobson et al. 2012; Umu et al. 2017). Bacteriocins have a number of advantages as antimicrobial substances. Unlike antibiotics suppressing metabolism and synthesis processes in bacteria, the action of bacteriocins is often accompanied by damage to the structures and death of the target cell, which reduces the possibility of microbial resistance. Besides, using bacteriocins is potentially advantageous due to their high biological activity (bacteriocins are efficacious in the nanomolar range) as well as low toxicity (except cytolysin). Unlike antibiotics, bacteriocins are completely metabolized in the human body, which determines their low toxicity. All this makes the use of these peptides more preferable than antibiotics in some cases (Mathur et al. 2013; Ming et al. 2015). Currently, a number of authors propose the use of BCN in combination with other antimicrobial drugs. There are studies on the synergistic effect of using BCN with antibiotic drugs (Zaslavskaya et al. 2019).

Conclusion

This study provides a comparative evaluation of the antimicrobial properties of LAB-derived postbiotics (BCN and EPS) and conventional antibiotics against Gram-negative Enterobacteriaceae, non-fermenting bacteria, and Gram-positive cocci. Conventional antibiotics demonstrated broad-spectrum efficacy against susceptible strains but showed predictable failures against multidrug-resistant (MDR) isolates, highlighting ongoing challenges in managing antibiotic resistance.

LAB-derived postbiotics, particularly BCN preparations from *L. rhamnosus* 2012 and *E. faecium* KE5, exhibited selective inhibitory activity against both susceptible and some resistant bacterial strains, whereas EPS-based postbiotics were largely ineffective. Postbiotics demonstrated strain-specific antimicrobial effects, with their activity being narrower than that of conventional antibiotics, but they offer advantages such as reduced risk of resistance development, potential synergy with host microbiota, and safety for immunocompromised individuals. Obtained data may be used for developing new probiotics against human pathogenic microflora. These findings represent preliminary *in vitro* observations, and further *in vivo* efficacy and toxicological studies are required to assess their potential therapeutic applications.

References

Aghamohammad S, Sakaki F, Haghighatshenas Z, Haj Agha Gholizadeh Khiavi E, Badmasti F, Rohani M (2025) Novel antimicrobial strategy: native postbiotics synergize with antibiotics to overcome resistance in *Klebsiella pneumoniae*. BMC Microbiology 25: e723. https://doi.org/10.1186/s12866-025-04474-7

Angelin J, Kavitha M (2020) Exopolysaccharides from probiotic bacteria and their health potential. International Journal of Biological Macromolecules 162: 853–865. https://doi.org/10.1016/j.ijbiomac.2020.06.190

Cuevas-González PF, Liceaga AM, Aguilar-Toalá JE (2020) Postbiotics and paraprobiotics: From concepts to applications. Food Research International 136: 109502. https://doi.org/10.1016/j.foodres.2020.109502

Daba GM, Elkhateeb W, Nour Soliman T, El-Dein AN, Zendo T (2024) Improving the functionality of yogurt after fortification with a synbiotic combination of a potential probiotic and bacteriocin-producing bacteria and *Hydnora abyssinica* phytosomes. Processes 12(4): 727. https://doi.org/10.3390/pr12040727

Dobson A, Cotter PD, Ross RP, Hill C (2012) Bacteriocin production: a probiotic trait? Applied and Environmental Microbiology 78(1): 1–6. https://doi.org/10.1128/AEM.05576-11

Grigoryan H, Marmaryan G, Soghomonyan T, Khoyetsyan L, Paloyan A, Tkhruni F, Karapetyan K, Hambardzumyan A (2025) Genetic and biochemical characterization of *Lacticaseibacillus rhamnosus* MDC 2012 and its potential in processing of raw pork meat. Bioactive Compounds in Health and Disease 8(11): 462–473. https://doi.org/10.31989/bchd.v8i11.1757

Horice B (2025) Antibiotic resistance trends in clinical isolates: A systematic review of global or regional patterns. International Journal of Biomedical and Clinical Research 3(4): 1–4. https://doi.org/10.59657/2997-6103.brs.25.059

Israyelyan A (2018) Investigation of lactic acid bacteria and their metabiotics isolated from milk of different domestic animals of Artsakh. MSc Thesis, 116 pp.

Israyelyan A, Balabekyan T, Sargsyan Z, Tkhruni F (2024) Effect of some metabiotics from strains of the genus *Enterococcus* on the growth of pathogenic bacteria that cause pneumonia. Biological Bulletin 51: 91–97. https://doi.org/10.31857/S1026347024010061

Jurášková D, Ribeiro SC, Silva CCG (2022) Exopolysaccharides produced by lactic acid bacteria: From biosynthesis to health-promoting properties. Foods 11: 156. https://doi.org/10.3390/foods11020156

Koohestani M, Moradi M, Tajik H, Badali A (2018) Effects of cell-free supernatant of *Lactobacillus acidophilus* LA5 and *Lactobacillus casei* 431 against planktonic form and biofilm of *Staphylococcus aureus*. Veterinary Research Forum 9(4): 301–306.

Kurnianto MA, Kusumaningrum HD, Lioe HN, Chasanah E (2021) Partial purification and characterization of bacteriocin-like inhibitory substances produced by *Streptomyces* sp. isolated from the gut of *Chanos chanos*. Biomedical Research International 2021: 7190152. https://doi.org/10.1155/2021/7190152

Li Z, Zhang Z, Qiao L, Zhao F, Xu X, Wang H, Tang X (2017) Characterization and bioactivities of the exopolysaccharide from a probiotic strain of *Lactobacillus plantarum* WLPL04. Journal of Dairy Science 100: 6895–6905. https://doi.org/10.3168/jds.2016-11944

M02-A13 (2023) Performance Standards for Antimicrobial Disk Susceptibility Tests M02-A13, 2023.

Manna A, Mondal R (2023) Bacteriocin-mediated food preservation in conjugation with silver nanoparticles: A green approach. Food Chemistry Advances 3: 100464. https://doi.org/10.1016/j.focha.2023.100464

Mathur H, O'Connor PM, Hill C, Cotter PD, Ross RP (2013) Analysis of anti-*Clostridium difficile* activity of thuricin CD, vancomycin, metronidazole, ramoplanin, and actagardine, both singly and in paired combinations. Antimicrobial Agents and Chemotherapy 57(6): 2882–2886. https://doi.org/10.1128/AAC.00261-13

Ming L, Zhang Q, Yang L, Huang JA (2015) Comparison of antibacterial effects between antimicrobial peptide and bacteriocins isolated from *Lactobacillus plantarum* on three common pathogenic bacteria. International Journal of Clinical and Experimental Medicine 8(4): 5806–5811.

Mokoena MP (2017) Lactic acid bacteria and their bacteriocins: Classification, biosynthesis and applications against uropathogens. Molecules 22: 1255. https://doi.org/10.3390/molecules22081255

Moradi M (2020) Postbiotics produced by lactic acid bacteria: The next frontier (review). https://doi.org/10.1111/1541-4337.12613

Nehal F, Sahnoun M, Smaoui S, Jaouadi B, Bejar S, Mohammed S (2019) Characterization, high production and antimicrobial activity of exopolysaccharides from *Lactococcus lactis* F-mou. Microbial Pathogenesis 132: 10–19. https://doi.org/10.1016/j.micpath.2019.04.018

Parente E, Brienza C, Moles M, Ricciardi A (1995) A comparison of methods for the measurement of bacteriocin activity. Journal of Microbiological Methods 22: 95–108. https://doi.org/10.1016/0167-7012(94)00068-I

Peng Z (2023) Progress in the application of food preservatives in food. Highlights in Science, Engineering and Technology 74: 1570–1575. https://doi.org/10.54097/05gj7k40

Rahman MM, Sazili AQ, Ahmad SA, Khalilah AK, Sarker MSK (2025) Inhibitory efficacy, production dynamics, and characterization of postbiotics of lactic acid bacteria. BMC Microbiology 25: 485. https://doi.org/10.1186/s12866-025-04123-z

Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, Sanders ME (2021) The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nature Reviews Gastroenterology & Hepatology 18: 649–667. https://doi.org/10.1038/s41575-021-00440-6

Seid M, Bayou B, Aklilu A, Tadesse D, Manilal A, Zakir A, Kulyta K, Kebede T, Alodaini HA, Idhayadhulla A (2025) Antimicrobial resistance patterns of WHO priority pathogens at a general hospital in southern Ethiopia during the COVID-19 pandemic, with particular reference to ESKAPE group isolates of surgical site infections. BMC Microbiology 25: 84. https://doi.org/10.1186/s12866-025-03783-1

Shafique B, Ranjha MMAN, Murtaza MA, Walayat N, Nawaz A, Khalid W, Mahmood S, Nadeem M, Manzoor MF, Ameer K, Aadil RM, Ibrahim SA (2023) Recent trends and applications of nanoencapsulated bacteriocins against microbes in food quality and safety. Microorganisms 11(1): 85. https://doi.org/10.3390/microorganisms11010085

Sørensen HM, Rochfort KD, Maye S, MacLeod G, Brabazon D, Loscher C, Freeland B (2022) Exopolysaccharides of lactic acid bacteria: Production, purification and health benefits towards functional food. Nutrients 14: 2938. https://doi.org/10.3390/nu14142938

Thuy TTD, Lu HF, Bregente CJB, Tu PC, Kao CY (2024) Characterization of the broad-spectrum antibacterial activity of bacteriocin-like inhibitory substance-producing probiotics isolated from fermented foods. BMC Microbiology 24: 85. https://doi.org/10.1186/s12866-024-03245-0

Tkhruni F, Saghyan AS, Balabekyan TsR, Karapetyan KJ, Khachatryan TV, Aghajanyan AE (2015) *Lactobacillus rhamnosus* BTK 20-12 bacteria strain, producing Rhamn 20-12 BCN1 and Rhamn 20-12 BCN2 bacteriocins. Patent RA No. 2924A.

Tkhruni F, Israyelyan A, Karapetyan K, Balabekyan T, Khachatryan L, Khachatryan T (2023) Comparative antimicrobial activity of some metabiotics synthesized by lactic acid bacteria. New Armenian Medical Journal 17: 98–109. https://doi.org/10.56936/18290825-2023.17.f-98

Tkhruni FN, Aghajanyan AE, Balabekyan TR, Khachatryan TV, Karapetyan KJ (2020) Characterization of bacteriocins from *Lactobacillus rhamnosus* BTK 20-12, a potential probiotic strain. Probiotics and Antimicrobial Proteins 12(2): 716–724. https://doi.org/10.1007/s12602-019-09569-y

Umu ÖCO, Rudi K, Diep DB (2017) Modulation of the gut microbiota by prebiotic fibres and bacteriocins. Microbiome and Ecological Health and Disease 28(1): 1348886. https://doi.org/10.1080/16512235.2017.1348886

Wang Y, Sholeh M, Yang LD, Shakourzadeh MZ, Beig M, Azizian K (2025) Global trends of ceftazidime–avibactam resistance in gram-negative bacteria: Systematic review and meta-analysis. Antimicrobial Resistance and Infection Control 14: 10. https://doi.org/10.1186/s13756-025-01518-5

WHO (2025) World Health Organization. WHO warns of widespread resistance to common antibiotics worldwide. News release. [13 Oct 2025]

Zapaśnik A, Sokołowska B, Bryła M (2022) Role of lactic acid bacteria in food preservation and safety. Foods 11(9): 1283. https://doi.org/10.3390/foods11091283

Zaslavskaya MI, Makhrova TV, Aleksandrova NA, Ignatova NI, Belova IV, Tochilina AG, Solovyeva IV (2019) Prospects for using bacteriocins of normal microbiota in antibacterial therapy (review). Modern Technologies in Medicine 11(3): 136–145. https://doi.org/10.17691/stm2019.11.3.17

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

Funding

This work was carried out within the framework of the base funding of the Institute of Armbiotechnology of the NAS of the Republic of Armenia and by the MESCS Science Committee of RA project scs 25RG-2I22.

Author contributions

Conceptualization: S.D., F.Tkh. Methodology: A.I., G.MA. Investigation: N.A., A.I. Data curation: N.A., A.I. Formal analysis: G.MA. Visualization: A.I., F.Tkh. Writing – original draft: S.D. and F.Tkh. Writing – review and editing: F.Tkh., S.D., G.MA.

Author ORCIDs

F. Tkhruni https://orcid.org/0000-0001-8649-6593
S. Danova https://orcid.org/0009-0006-3921-3785
G. Melik-Andreasyan https://orcid.org/0000-0002-5796-8077
N. Aleksanyan https://orcid.org/0000-0003-2472-9990
A. Israyelyan https://orcid.org/0000-0002-6789-9853

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

Supplementary material 1

Supplementary figures

Authors: Flora Tkhruni, Svetla Danova, Gayane Melik-Andreasyan, Naira Aleksanyan, Arevik Israyelyan
Data type: zip
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/pharmacia.73.e189800.suppl1

Supplementary material 2

Supplementary tables

Authors: Flora Tkhruni, Svetla Danova, Gayane Melik-Andreasyan, Naira Aleksanyan, Arevik Israyelyan
Data type: docx
Explanation note: The detailed data on antibiotic and postbiotic activity against each individual pathogen.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/pharmacia.73.e189800.suppl2