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Research Article

In-vitro antifungal and anticlostridial activity of lyophilized postbiotics from *Lactiplantibacillus plantarum, Lacticaseibacillus casei,* and *Lactobacillus helveticus* strains

Lactiplantibacillus plantarum, Lacticaseibacillus casei ve *Lactobacillus helveticus* Suşlarından Elde Edilen Liyofilize Postbiyotiklerin İn-Vitro Antifungal ve Anticlostridial Etkinliklerinin Belirlenmesi

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Abstract

Molds and *Clostridium* species are significant microorganisms responsible for food spoilage and are also considered potential threats to public health. The aim of this study is to determine the antifungal and anticlostridial activities of lyophilized postbiotics obtained from *Lactobacillus* spp. (*Lb. plantarum*, *Lb. casei*, and *Lb. helveticus*) and to evaluate their pH and titratable acidity values. In addition, the in vitro antimicrobial effects against strains of *Aspergillus parasiticus, Penicillium crysogenum, Clostridium sporogenes,* and *Clostridium tepidum* were investigated (minimum inhibitory (MIC), minimum bactericidal (MBC)/minimum fungicidal concentration (MFC), and zone of inhibition diameters). The pH values of the postbiotics obtained from *Lactobacillus* spp. ranged from 3.98 to 4.58, while their titratable acidity values ranged from 1.28 - 9.38 g lactic acid/100 mL. The MIC, MBC/MFC, and inhibition zone values of *Lactobacillus* spp. against the specified mould and *Clostridium* strains were found to range from 12.5 mg/mL, 50–100 mg/mL, and 17.36–24.25 mm, respectively. In conclusion, lyophilized postbiotics derived from *Lactobacillus* strains demonstrated strong antifungal and anticlostridial activities, with their efficacy increasing in a concentration-dependent manner.

Keywords: Antimicrobial activity; Lactic acid bacteria; Lyophilization; Postbiotics

Öz

Küfler ve *Clostridium* türleri, gıda bozulmasından sorumlu önemli mikroorganizmalar olup aynı zamanda halk sağlığı için potansiyel bir risk olarak kabul edilmektedir. Bu çalışmanın amacı, *Lactobacillus* spp. (*Lb. plantarum*, *Lb. casei* ve *Lb. helveticus*) kullanılarak elde edilen liyofilize postbiyotiklerin antifungal ve antiklostridyal aktivitelerini belirlemek ve ayrıca pH ile titre edilebilir asitlik değerlerini değerlendirmektir. Ayrıca *Aspergillus parasiticus, Penicillium crysogenum, Clostridium sporogenes* ve *Clostridium tepidum* suşlarına karşı in vitro antimikrobiyal etkileri (minimum inhibisyon (MİK), minimum bakterisidal (MBK)/minimum fungusidal konsantrasyonu (MFK) ve inhibisyon zon çapları) incelenmiştir. *Lactiplantibacillus* türlerinden elde edilen postbiyotiklerin pH değerleri 3,98 ile 4,58 arasında değişirken, titrasyonla belirlenen asitlik değerlerinin 100 mL 'de 1,28 ile 9,38 g laktik asit arasında değiştiği bulunmuştur. *Lactobacillus* suşlarının belirtilen küf ve *Clostridium* suşlarına karşı MİK, MBK/MFK ve inhibisyon zon değerlerinin sırasıyla 12.5 mg/mL, 50-100 mg/mL ve 17.36-24.25 mm (400 mg/mL) arasında olduğu belirlenmiştir. Sonuç olarak, *Lactobacillus* suşlarından elde edilen liyofilize postbiyotiklerin güçlü antifungal ve antiklostridiyal aktivite sergilediği ve bu etkinliğin konsantrasyona bağlı olarak arttığı ortaya konulmuştur.

Anahtar Kelimeler: Antimikrobiyal aktivite; Laktik asit bakterisi; Liyofilizasyon; Postbiyotik

Introduction

In recent years, the demand of consumers for clean-label foods has result to the common use of natural biopreservatives, such as lactic acid bacteria (Trymers et al., 2026). For many centuries, lactic acid bacteria (LAB) have been used in food for the purpose of bio-preservation. These bacteria are GRAS and enhance food safety and quality, in part due to the antimicrobial and antioxidant properties of their bioactive metabolites (postbiotics) (Incili et al., 2025a; Ozma et al., 2026). In addition, LAB and their metabolites have a wide range of applications, from biotechnology and healthcare to sustainable packaging systems, and from cellular agriculture to biofilm control (Incili et al., 2025a; Ozma et al., 2026; Trymers et al., 2026). Postbiotics are non-living microorganisms and/or their by-products that provide health benefits. Postbiotics possess a variety of bioactive properties, including antimicrobial, antifungal, antioxidant, anti-inflammatory, and anti-carcinogenic effects due to the presence of its metabolites, such as organic acids, enzymes, bacteriocins, and exopolysaccharides (Akgöl et al., 2025, Tutuk et al., 2026). The lyophilization transform postbiotics more stable and increases their bioactive components (e.g. organic acids, phenolic compounds, peptides, and other metabolites) by up to 20-times, increasing their antimicrobial activity (Tutuk et al., 2026). Studies have shown that removing the liquid portion of postbiotics and concentrating them synergistically increases their inhibitory effect on foodborne pathogens (Incili et al., 2023b; Trymers et al., 2026;Tutuk et al., 2026).

Molds are biological hazards that negatively affect the texture, colour, taste and odour of foods, shortening their shelf life and presenting serious threats to both food safety and public health. In particular, aflatoxin derivatives produced by fungal strains, such as *Aspergillus parasiticus* and *Aspergillus flavus* pose a serious threat to human health and exhibit carcinogenic effects. Futhermore, Other toxins produced by these molds can also cause foodborne illnesses (Incili et al., 2025b; Tutuk et al., 2026; Davarzani et al., 2025). *Clostridium* spp., particularly *Clostridium botulinum* and *Clostridium perfringens*, are pathogens that pose a serious global public health threat and are among the most prevalent causes of foodborne illness (Davarzani et al., 2025; Kyrylenko et al., 2023). A number of studies have indicated that postbiotics exhibit potent antifungal and anticlostridial activity (Ozma et al., 2024; Demirbaş et al., 2022; Incili et al.,2025a; Kaya et al., 2025; Mohammadi et al., 2022; Ramazanidoroh et al.,2025; Trymers et al.,2026; Tutuk et al., 2026). The potent antifungal and anticlostridial properties of postbiotics can be attributed to the certain bioactive compounds, including organic acids, fatty acids, and bacteriocins (Demirbaş et al., 2022; Rahman et al., 2026;Trymers et al., 2026; Tutuk et al., 2026). However, despite these findings, limited information is available regarding the antifungal and anticlostridial activities of lyophilized postbiotics derived from different *Lactobacillus* strains against selected food spoilage and food safety-related microorganisms. The aim of this study is to investigate *Lactobacillus* strains (*Lactiplantibacillus plantarum ATCC 14917, Lacticaseibacillus casei ATCC 393,* and *Lactobacillus helveticus ATCC 15009*) to determine the *in vitro* antifungal and anticlostridial activities, as well as the pH and titratable acidity values of the lyophilized postbiotics.

Materials and Methods

In this study, we used *Lactiplantibacillus plantarum* ATCC 14917, *Lacticaseibacillus casei* ATCC 393, and *Lactobacillus helveticus* ATCC 15009, as well as strains of *Aspergillus parasiticus* NRRL 2999, *Penicillium*

crysogenum ATCC 100106, *Clostridium sporogenes*, and *Clostridium tepidum* (field strains isolated by Demirbaş et al., 2022) from our department's collection.

Production of Lyophilized Postbiotics

For the activation of *L. plantarum* ATCC 14917, *L. casei* ATCC 393, and *L. helveticus* ATCC 15009 strains, 100 µL of the strains stored in 20% glycerol + de Man, Rogosa, and Sharpe (MRS) Broth at −18 °C were transferred to 9.9 mL of MRS medium, and incubated at 37 °C for 24 h. Afterwards, the activated bacteria were added to the same medium at a 1% concentration and incubated for another 48 h. Subsequently, the bacteria were inactivated by being kept at 85°C for 40 min. The inactivated bacteria were transferred to sterile trays, frozen at -20°C for 24 h, and then lyophilized at -55/-60°C and 4.6 x 10^{-2} bar pressure for 18-24 h (Toros TRS 2/2V, Teknosem, Türkiye). Lyophilized postbiotics were stored in sterile jars at 4°C until use (Tutuk et al., 2026).

Preparation of Experimental Groups and Inoculum

The groups were divided into 12 subgroups, with 3×4 subgroups prepared for each bacterial culture: 1× (50 mg/mL), 2× (100 mg/mL), 4× (200 mg/mL), and 8× (400 mg/mL).

Following the inoculation of the mold strains into Sabouraud dextrose broth, the samples were subjected to incubation at 25 °C for a period of 48 hours. Then, 100 µL of the solution was placed on the surface of SD agar (Biokar, France), and the plates were then incubated at the same temperature for a 5-day period. Subsequently, 10 mL of phosphate buffer solution was dispensed onto the plate surface, and the molds were collected from the agar surface with care. Thereafter, the collected cells were filtered with a sterile material to separate the mycelia, and the filtrate was then employed as a stock mold inoculum (Tutuk et al., 2026).

Clostridium species, Brain Heart Infusion (BHIB) Broth was used to prepare the inoculum. For this aim, 100 µL of the *Clostridium* strain was added to 9.9 mL of BHIB and incubated under anaerobic conditions at 37 °C for 24 hours. Following incubation, the samples were subjected to centrifugation at 4200 × g for 10 minutes, and the resultant supernantants were removed. The pellets were resuspended in 2 mL of 0.1% peptone water. Thereafter, the absorbances of the pellets were adjusted to 1.0 at 600 nm. Thus, the concentration of each bacterium was ~ 10^8 CFU/mL. In order to conduct in vitro antimicrobial activity tests using mold and *Clostridium* strains, tenfold dilutions were prepared (Akgöl et al., 2025; Demirbaş et al., 2022).

pH and Titratable Acidity

The pH values of the postbiotics were measured using a digital pH meter (HI 11310; Hanna Instruments, USA). The titratable acidity was determined using the method described by Serter et al. (2024). In summary, after the addition of a few drops of phenolphthalein indicator to 10 mL of postbiotic taken from the prepared stock solutions, the titration was performed with 0.25 N NaOH (Merck, Emplura, Darmstadt, Germany) until a pink colour appeared. For the aim of determining the amount of NaOH required for 100 mL of postbiotic, the amount of 0.25 N NaOH used was multiplied by 10, and the resulting value was calculated as the percentage by weight of lactic acid.

In Vitro Antifungal and Anticlostridial Activity Assays

The antifungal and anticlostridial activities of lyophilized postbiotics against the strains *Aspergillus parasiticus* NRRL 2999, *Penicillium crysogenum* ATCC 100106, *Clostridium sporogenes*, and *Clostridium tepidum* have been determined. The broth dilution method was used to determine the MIC values. After preparing SDB for mold species and BHIB for *Clostridium* species, the broths were added to the sterile tubes at a volume of 4 mL in the first tube and 2 mL in all subsequent tubes. Following this, 100 mg/mL of dried postbiotic was added to the first tube and vortexed until homogeneous. A volume of 2 mL was drawn from the first tube and transferred to the second tube, thereby performing a two fold dilution. In this procedure, two dilutions were performed in each tube, resulting in a total of at least eight dilution steps. A volume of 2 mL was transferred from the final dilution tube and disposed of, and the volumes in all tubes were made equivalent. A volume of 100 µL of the bacterial inoculum, as prepared above, was added to all tubes that had been diluted in two-fold series. The tubes were then incubated for 3 days at 25 °C for molds and for 24 h at 37 °C for *Clostridium* species. Following the incubation process, the final tube that exhibited no turbidity (i.e. clarity) was identified as the MIC value (Incili et al., 2023b; Tutuk et al., 2026). 10 µL of all tubes showing no visible growth in the MIC test was spread onto SDA and Brain Heart Infusion Agar (BHIA) plates, and the plates were incubated at 25 °C and 37 °C, respectively. After the incubation period, the lowest concentration that exhibits no colony formation was identified as the MFC and MBC. To determine the inhibition zones, 100 µL of bacterial culture was taken from the prepared dilutions and spread onto the surface of pre-prepared SDA and BHIA (15 mL); the petri dishes were then kept at room temperature for 10 min to allow the microorganisms to adhere. Next, the lyophilized postbiotics were prepared at concentrations of 1× (50 mg/mL), 2× (100 mg/mL), 4× (200 mg/mL), and 8× (400 mg/mL); 20 µL of each solution was dropped onto the surface directly petri dishes and incubated for 5 days at 25 °C and 24 h at 37 °C, respectively. The diameters of the clear zones post-incubation were measured using a digital micrometer (Akgöl et al., 2025; Ozma et al., 2024; Tutuk et al., 2026).

Ethical Approval

For this study, ethical approval was obtained from the Fırat University Ethics Committee for Non-Interventional Research via its decision dated 8 April 2026, No. 46538.

Statistical Analysis

Statistical analyses were performed using SPSS software (IBM SPSS, version 21.0, IBM Corporation, USA). For multiple comparisons, analysis of variance (ANOVA) was used with a post hoc Tukey test, and the significance level was defined as $p < 0.05$.

Results

The pH values and titratable acidity levels (expressed as g lactic acid/100 mL) of the lyophilized postbiotics obtained from *Lactiplantibacillus plantarum* ATCC 14917, *Lacticaseibacillus casei* ATCC 393, and *Lactobacillus helveticus* ATCC 15009 cultures are presented in Table 1. As shown in Table 1, the pH values of the postbiotics obtained from these three different lactic acid bacteria cultures decreased as the postbiotic concentrations (1x, 2x, 4x, and 8x) increased. Nevertheless, when prepared at the same concentration, the pH values of the postbiotics derived from *Lb. plantarum* and *Lb. helveticus* were not significantly different ($p>0.05$), whereas the postbiotic obtained from the *Lb. casei* culture exhibited a significantly lower pH value

($p<0.05$).The lowest pH value was found to be 3.98 ± 0.03 in the 8xc group ($p<0.05$). When the acidity levels of postbiotics derived from *Lb. plantarum, Lb. casei*, and *Lb. helveticus* were examined by titration, it was demonstrated that acidity increased in a concentration-dependent manner (1x, 2x, 4x, and 8x) ($p<0.05$). The highest titratable acidity value was determined to be 9.38 ± 0.34% g/L in the 8xc group, which was obtained from the *Lb. casei* (see Table 1, $p<0.05$).

Table 1. pH and titratable acidity of lyophilized postbiotics obtained from *Lactobacillus* spp. Strains (Mean ± S.D.)

Groups	pH	Acidity (g lactic acid/100 mL)
1×p	4.58 ± 0.02^{a}	1.58 ± 0.23^{gh}
2×p	4.53 ± 0.02^{b}	2.55 ± 0.47^{fg}
4×p	4.46 ± 0.01^{c}	4.35 ± 0.26^{cd}
8×p	4.42 ± 0.01^{d}	7.76 ± 0.16^{b}
1×c	4.24 ± 0.01^{e}	1.80 ± 0.39^{gh}
2×c	4.20 ± 0.01^{f}	3.23 ± 0.34^{ef}
4×c	4.09 ± 0.02^{g}	4.95 ± 0.23^{c}
8×c	3.98 ± 0.03^{h}	9.38 ± 0.34^{a}
1×h	4.57 ± 0.01^{a}	1.28 ± 0.13^{h}
2×h	4.53 ± 0.01^{b}	2.40 ± 0.34^{fg}
4×h	4.48 ± 0.01^{c}	3.71 ± 0.48^{de}
8×h	4.42 ± 0.02^{d}	7.09 ± 0.48^{b}

Values with different superscripts in the same column are statistically significant ($p<0.05$). 1×: 50 mg/mL, 2×: 100 mg/mL, 4×:200 mg/mL, 8×: 400 mg/mL p: postbiotic of *Lactiplantibacillus plantarum*, c:postbiotic of *Lactocaseibacillus casei* h: postbiotic of *Lactobacillus helveticus*

The MIC and MFC values of postbiotics derived from *Lb. plantarum*, *Lb. casei*, and *Lb. helveticus* against *Aspergillus parasiticus* NRRL 2999 and *Penicillium chrysogenum* ATCC 100106 were determined to be 12.5 mg/mL and 100 mg/mL, respectively, for all three cultures. Against *Clostridium sporogenes* and *Clostridium tepidum*, the MIC/MBC values were found to be 12.5/100 mg/mL, 12.5/100 mg/mL, and 12.5/50 mg/mL for the postbiotics derived from *Lb. plantarum*, *Lb. casei*, and *Lb. helveticus*, respectively (Table 2).

Table 2. Minimum inhibitory and minimum bactericidal/fungicidal concentrations of lyophilized postbiotics derived from *Lactobacillus* spp. strains against mold and *Clostridium* spp. (Mean ± Standard Deviation) (mg/mL)

Groups	*A.parasiticus* NRRL 2999		*P.crysogenum* ATCC 100106		*Cl. sporogenes*		*Cl. tepidum*	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MBC**	**MIC**	**MBC**
***Lb. plantarum* ATCC 14917**	12.5±0.0	100.0±0.0	12.5±0.0	100.0±0.0	12.5±0.0	100.0±0.0	12.5±0.0	100.0±0.0
***Lb. casei* ATCC 393**	12.5±0.0	100.0±0.0	12.5±0.0	100.0±0.0	12.5±0.0	100.0±0.0	12.5±0.0	100.0±0.0
***Lb. helveticus* ATCC 15009**	12.5±0.0	100.0±0.0	12.5±0.0	100.0±0.0	12.5±0.0	100.0±0.0	12.5±0.0	50.0±0.0

The inhibition zone diameters of the postbiotics obtained from these three *Lactobacillus* spp. cultures against the mold and *Clostridium* spp. used in the study are shown in Figure 1. As shown in Figure 1, it was observed that as the concentration of postbiotics derived from *Lactobacillus* spp. cultures increased (1×, 2×, 4×, and 8×), the diameters of the inhibition zones also increased in parallel ($p<0.05$). The highest inhibition zone diameters against *Aspergillus parasiticus, Penicillium crysogenum,* and *Clostridium sporogenes* were determined in the 8xp group, with values of 24.25 ± 0.55, 21.24 ± 0.51, and 23.37 ± 0.39 mm, respectively ($p<0.05$). The highest zone against the *Clostridium tepidum* strain was observed in the 8xc group, at 20.16 ± 0.52 mm ($p<0.05$).

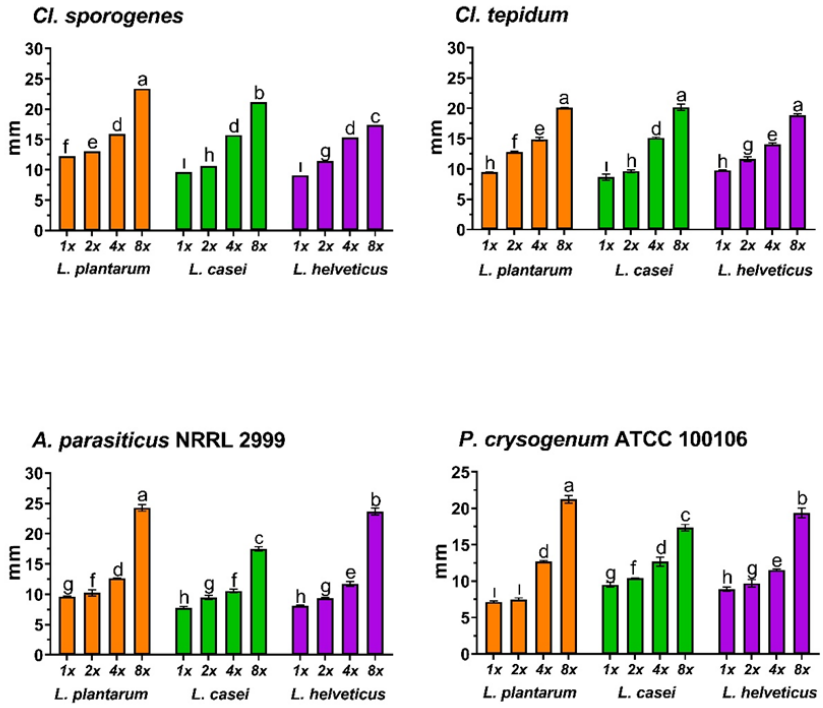

Figure 1. Inhibition zone diameters of lyophilized postbiotics obtained from *Lactobacillus* spp. strains (Mean ± SD, mm) (1x: 50 mg/mL, 2×: 100 mg/mL, 4×: 200 mg/mL, 8×: 400 mg/mL). a–ı: The mean values with different letters are significantly different ($p<0.05$).

Discussion

Titratable acidity is measured by neutralising the total soluble acidity with a base. This value serves as a parameter indicating the production of organic acids by lactic acid bacteria (Çobur et al., 2025). Postbiotics contain high levels of various organic acids, e.g. lactic, acetic and propionic, which contribute to the antimicrobial effect by lowering the pH and disrupting the bacterial cell membrane. As the pH decreases, the titratable acidity increases (Incili et al., 2025a,b). The pH and titratable acidity values of the postbiotics obtained from *Lactobacillus* spp. strains are presented in Table 1. The study's findings indicated that as postbiotic concentrations increased, there was a decrease in pH while titratable acidity showed an increase. It was determined that the lowest pH value and the highest titratable acidity value were found in the 8xc group (3.98 ± 0.03 and 9.38 ± 0.34)($p<0.05$). In accordance with the findings of this study, a research article by Incili et al. (2025b) determined that the postbiotic derived from a *Latilactobacillus sakei*/*Staphylococcus xylosus* culture exhibited a lower pH value and a higher titratable acidity value in comparison to the postbiotic derived from a *Pediococcus acidilactici* culture *(*$p<0.05$*).* In a another study, cultures of various lactic acid bacteria (*L. plantarum, L. sakei, L. curvatus, L. paracasei, L. rhamnosus, L. fermentum, L. reuteri, P. acidilactici*, and *L. delburuckii subsp. bulgaricus*) were used, and it was observed that the pH value of the *L. sakei* postbiotic was lower compared to other postbiotics, while the highest titratable acid levels were found in the postbiotics derived from *L. plantarum*, *L. sakei*, and *L. curvatus (p<0.05)* (Sezen et al. 2024). The differences in pH and titratable acidity values are thought to be due to the type of bacteria and fermentation method, bacterial growth conditions, the culture medium, incubation time, concentration process, and the organic acids in the sample (Çobur et al.,2025; Incili et al., 2025a,b; Sezen et al., 2024). Postbiotics have a high antimicrobial effect due to metabolites such as organic acids, bacteriocins, short-chain fatty acids, polyphenols and volatile compounds (Dawarzani et al., 2025; Trymers et al., 2026). The present study examined the in vitro antimicrobial properties of postbiotics obtained from *Lactobacillus* spp. strains against mold and *Clostridium* species. In the present study, it was determined that the postbiotics derived from *Lb. plantarum, Lb. casei*, and *Lb. helveticus* had MIC values of 12.5 mg/mL and MBC/MFC values of 100 mg/mL against mold and *Clostridium* strains, respectively (the MBC value of *Lb. helveticus* against *C. tepidum* was 50 mg/mL). Postbiotics derived from *Lactobacillus* spp. cultures showed the largest inhibition zone diameters against both mold and *Clostridium* spp. in the 8xp group. In addition, it was determined that as the concentration of the postbiotics increased, so too did their antimicrobial activity (Figure 1). In our previous study used a lyophilized postbiotic from a *Lb. plantarum* culture and found MIC, MFC, and inhibition zone values against *A. parasiticus* of 12.5, 100 µg/ml, and 16.47 mm (Tutuk et al., 2026). Abdel-Nasser et al. (2023) determined that the inhibition zone diameter of the postbiotic obtained from the *Lactobacillus rhamnosus* ATCC-7469 culture against *Aspergillus* species ranged from 17.7 ± 0.5 to 21.3 ± 0.6 mm. Additionally, according to Ozma et al. (2024), the MIC values and inhibition zone diameter measurements of postbiotics derived from *B. bifidum* and *Lb. plantarum* cultures against *C. difficile* ATCC 43255 were determined to be 2.5 mg/mL and 5 mg/mL, 13 mm and 10 mm, respectively. In a study conducted by Kaya et al. (2025), postbiotics obtained from various LAB cultures (*L. plantarum Y48, L. hordei SK-6,* and *Lp. plantarum VB-29*) demonstrated strong antifungal activity against the molds *P. expansum, P. concentricum, P. chrysogenum, Fusarium* spp., *Alternaria alternata, A. parasiticus,* and *A. niger*. The observed variations among the studies are hypothesised to be caused by multiple factors, including the type of LAB used, the growth medium and culture conditions, the amount of metabolites contained in the postbiotic,

the method of postbiotic production (liquid/solid, etc.), concentration, and the susceptibility of the target microorganism, as well as potential contributions from decreases in pH and increases in titratable acidity in conjunction with other bioactive postbiotic components (Çobur et al., 2025; Dawarzani et al., 2025; Incili et al., 2025a;Trymers et al., 2026; Tutuk et al.,2026).

Conclusion

In conclusion, the present study demonstrated that lyophilized postbiotics derived from *Lactobacillus* spp. (*Lb. plantarum, Lb. casei,* and *Lb. helveticus*) exhibit strong, concentration-dependent in vitro antimicrobial activity against food spoilage and food safety-related mold and *Clostridium* strains. These effects were confirmed by MIC, MBC/MFC, and inhibition zone assays and were associated with acidic pH and appreciable titratable acidity, suggesting a role for organic acid production and other bioactive metabolites in the observed antimicrobial activity. Overall, these findings highlight the potential of lyophilized postbiotics as natural biopreservatives for improving food safety, extending shelf life, and enhancing food quality.

Conflict of interest: The authors declare that they have no competing interests.

For Studies with Ethics Committee Approval: *For this study, ethical approval was obtained from the Fırat University Ethics Committee for Non-Interventional Research via its decision dated 8 April 2026, No. 46538*.

Obtaining Informed Consent: "*All participants provided written informed consent prior to data collection.*"

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Data Availability Statement: Authors are encouraged to share their research data. If applicable, one of the following statements may be used: "The datasets used in this study are available from the study's corresponding author upon reasonable request. *Corresponding Author Contact: makgol@firat.edu.tr.*"

CRediT author statement: MA: Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation, Writing- Reviewing and Editing. SB: Visualization, Investigation. GKİ: Data curation, Supervision, Software, Validation. All authors have read and agreed to the published version of the manuscript.

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