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THE ANTIBACTERIAL ACTIVITY OF WHOLE-CELL POSTBIOTICS DERIVED FROM A TRADITIONAL TURKISH DAIRY PRODUCT, MASTIK, AGAINST FOODBORNE PATHOGENS

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ABSTRACT

Traditional fermented dairy products serve as a rich reservoir of functionally active lactic acid bacteria (LAB), which produce various bioactive metabolites with potential antimicrobial properties. Mastik, a distinctive handmade dairy product, is traditionally produced in the Elazığ, Tunceli, and Erzincan regions of Turkey. It is characterized by a multi-stage fermentation and maturation process that supports a complex microbial ecosystem. Despite its rich microbiological heritage, the antibacterial potential of postbiotics derived from mastik remains largely unexplored. Bacteria isolated from mastik samples were incubated in MRS broth at 37 °C for 48 hours to produce postbiotics. Bacterial cells were inactivated by heat treatment at 70 °C for 30 minutes to obtain whole-cell postbiotic. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the prepared postbiotics against *Salmonella* Typhimurium (ATCC 14028), *Listeria monocytogenes* (RSKK 472), and *Escherichia coli* O157:H7 (ATCC 43894) were determined by the liquid microdilution method. According to the analysis results, the MIC value was determined as 15 mg/mL for all pathogens tested. The MBC values were found to be 60 mg/mL for *S.* Typhimurium and *L. monocytogenes*, and 30 mg/mL for *E. coli* O157:H7. The findings indicate that the postbiotic provides broad-spectrum inhibition against both Gram-positive and Gram-

negative bacteria. In conclusion, it has been demonstrated that traditional fermented products like mastik, owing to their rich microbial ecosystems, hold significant potential for the development of natural bioprotective agents. It is recommended that the active compounds of the postbiotics obtained be more thoroughly characterized and validated in food model systems.
Key Words: Mastik, postbiotic, antibacterial activity, MIC, MBC, food safety

GELENEKSEL BİR TÜRK SÜT ÜRÜNÜ OLAN MASTİKTEN ELDE EDİLEN TAM HÜCRELİ POSTBİYOTİKLERİN GIDA KAYNAKLI PATOJENLERE KARŞI ANTİBAKTERİYEL ETKİSİ

Özet

Geleneksel fermente süt ürünleri, potansiyel antimikrobiyal özelliklere sahip çeşitli biyoaktif metabolitler üreten, fonksiyonel olarak aktif laktik asit bakterilerinin (LAB) zengin bir kaynağıdır. Elle yapılan özgün bir süt ürünü olan mastik, Türkiye'nin Elazığ, Tunceli ve Erzincan bölgelerinde geleneksel olarak üretilmektedir. Bu ürün, karmaşık bir mikrobiyal ekosistemi destekleyen çok aşamalı bir fermantasyon ve olgunlaşma süreciyle karakterize edilir. Zengin mikrobiyolojik mirasına rağmen, mastikten elde edilen postbiyotiklerin antibakteriyel potansiyeli büyük ölçüde keşfedilmemiştir. Postbiyotik üretmek için mastik örneklerinden izole edilen bakteriler, 37 °C'de 48 saat boyunca MRS besiyerinde inkübe edildi. Tam hücreli postbiyotik elde etmek için bakteri hücreleri, 70 °C'de 30 dakika boyunca ısıl işlemle inaktive edildi. Hazırlanan postbiyotiklerin Salmonella Typhimurium (ATCC 14028), Listeria monocytogenes (RSKK 472) ve Escherichia coli O157:H7 (ATCC 43894) suşlarına karşı minimum inhibitör konsantrasyon (MIC) ve minimum bakterisidal konsantrasyon (MBC) değerleri, sıvı mikrodilüsyon yöntemi ile belirlenmiştir. Analiz sonuçlarına göre, test edilen tüm patojenler için MIC değeri 15 mg/mL olarak belirlendi. MBC değerlerinin S. Typhimurium ve L. monocytogenes için 60 mg/mL, E. coli O157:H7 için ise 30 mg/mL olduğu tespit edildi. Bulgular, postbiyotiğin hem Gram-pozitif hem de Gram-negatif bakterilere karşı geniş spektrumlu inhibisyon sağladığını göstermektedir. Sonuç olarak, mastik gibi geleneksel fermente ürünlerin, zengin mikrobiyal ekosistemleri sayesinde doğal biyokoruyucu ajanların geliştirilmesinde önemli bir potansiyele sahip olduğu gösterilmiştir. Elde edilen postbiyotiklerin aktif bileşiklerinin gıda model sistemlerinde daha kapsamlı bir şekilde karakterize edilmesi ve doğrulanması önerilmektedir.

Anahtar Kelimeler: Mastik, postbiyotik, antibakteriyel aktivite, MIC, MBC, gıda güvenliği

INTRODUCTION

Fermented dairy products are recognized as significant functional foods due to their nutritional value and contribution to food safety, attributed to their rich microbial diversity and bioactive components. Notably, lactic acid bacteria (LAB), which are predominantly present in these products, generate various antimicrobial compounds during fermentation, thereby inhibiting the proliferation of foodborne pathogens. Among the compounds synthesized by LAB are organic acids, bacteriocin-like inhibitory substances, and other metabolites, all of which have been documented to possess broad-spectrum antimicrobial activity (Mishra et al., 2024; Rahman et al., 2025).

Recent studies have demonstrated that the health and food safety benefits of microorganisms extend beyond their live cell form; the postbiotic form, comprising inactivated

cells and metabolites of these microorganisms, also exhibits significant biological activities (Vinderola et al., 2022; Mishra et al., 2024). Postbiotics are defined as components consisting of inactivated microorganisms or their metabolites that confer beneficial effects on host health, offering particular advantages over probiotics in terms of safety, stability, and shelf life (Aydemir et al., 2025; Demircioğlu et al., 2026).

Recent investigations into the antimicrobial properties of postbiotics derived from lactic acid bacteria (LAB) strains isolated from fermented foods have demonstrated their efficacy against both Gram-positive and Gram-negative pathogens (Chang et al., 2021). Notably, postbiotics obtained from species such as *Lactobacillus plantarum*, *L. acidophilus*, and *L. rhamnosus* have been reported to exhibit significant inhibitory activity against major pathogens, including *E. coli*, *S.* Typhimurium, and *Staphylococcus* aureus (Rahman et al., 2025). Similarly, postbiotics produced from LAB strains isolated from traditional dairy products have been shown to significantly reduce *E. coli* counts in model food systems, indicating their bioprotective potential (Tariq et al., 2025).

Traditional and artisanal dairy products are considered valuable sources for the discovery of novel functional microorganisms, as they contain more complex and unique microbial communities compared to industrial products. The natural microbiota present in these products, particularly during fermentation and ripening processes, contributes to the formation of metabolites with antimicrobial activity (Tariq et al., 2025). Consequently, exploring the microbial potential of local and traditional dairy products is increasingly important for the development of next-generation natural bioprotective agents.

Mastik, a traditional dairy product originating from Turkey and produced using conventional methods, has been the focus of limited scholarly investigation. This product potentially harbors a rich microbial ecosystem due to its extensive fermentation and maturation processes. Mastik is a distinctive fermented product, predominantly produced during the autumn months in the Elazığ, Tunceli, and Erzincan regions of Turkey, and is notable for its long-term storability. The production process involves the mechanical processing of milk to separate the fat phase, followed by heating and initiating controlled fermentation through the addition of yogurt. The fermentation process, lasting approximately 48 hours, is succeeded by mechanical mixing and straining to separate the product into its phases. The resultant liquid portion undergoes further heat treatment until it achieves a thick consistency. In the final stage, the product is allowed to mature at room temperature for an extended duration, thereby acquiring its characteristic texture and aromatic properties (Figure 1). This multi-stage production process significantly contributes to the rich and unique microbial composition of mastik. However, the literature on the microbial composition of specific products like mastik, particularly concerning the antibacterial effects of postbiotics derived from these products, remains sparse. This paucity of information underscores the need for scientific research into these products.

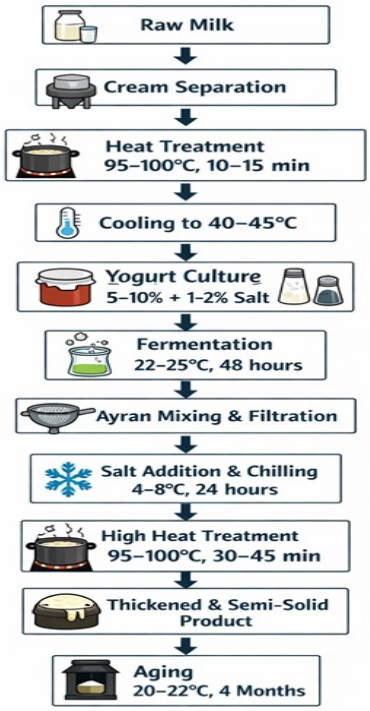

Figure 1: Mastik production workflow diagram

This study aims to produce postbiotics utilizing bacteria isolated from mastic, a traditional dairy product, and to assess the antibacterial efficacy of the resulting postbiotics against prominent foodborne pathogens, including *S.* Typhimurium, *L. monocytogenes*, and *E.coli* O157:H7. This evaluation will employ the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methodologies.

MATERIALS AND METHODS

The Postbiotic Production Stage From Mastik

Mastik was procured from producers in Elazığ province who employ traditional methods (Figure 2). From the mastic transported to the laboratory under a cold chain, 10 mL was extracted under aseptic conditions and transferred into tubes containing 90 mL of Man, Rogosa, and Sharpe broth (MRS broth). The bacteria were pre-enriched by incubating at 37°C for 24 hours. Following incubation, the enriched culture was inoculated onto MRS agar medium using the streak plate method and incubated at 37°C for an additional 24 hours to facilitate the development of isolated colonies. Fresh and well-developed colonies formed at the conclusion of incubation were collected and inoculated into tubes containing 9 mL MRS broth, and postbiotic production was conducted by incubating at 37°C for 48 hours. The postbiotic was produced with modifications to the method described in Aydemir (2025). To verify bacterial density, samples from the culture were inoculated onto MRS agar medium as a control, confirming the bacterial density to be approximately 10^8 CFU/mL. After the 48-hour production

period, bacterial cells were inactivated to obtain the postbiotic. For this purpose, the culture suspension underwent heat treatment at 70°C for 30 minutes to inactivate the bacteria. The preparation containing whole-cell postbiotic obtained was stored at 4°C for subsequent experimental analyses.

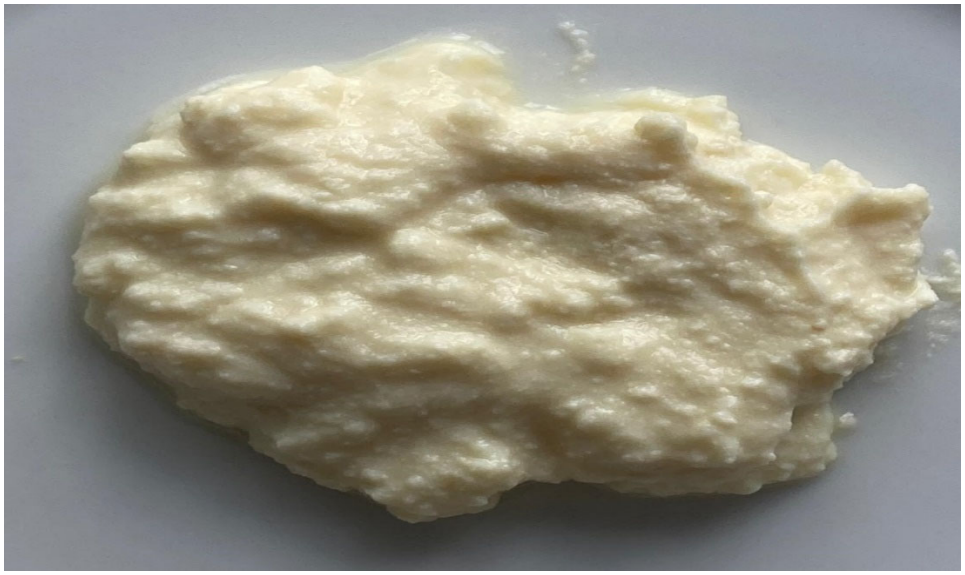

Figure 2. A traditional mastic product used as a source in postbiotic production

Determination of the Antibacterial Activity of Postbiotic

The antibacterial efficacy of the postbiotic was assessed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The study utilized Salmonella Typhimurium (ATCC 14028), Listeria monocytogenes (RSKK 472), and Escherichia coli O157:H7 (ATCC 43894) as test organisms.

MIC determination was conducted using the broth microdilution method, with modifications based on the Clinical and Laboratory Standards Institute (CLSI) protocol. Tryptic Soy Broth served as the culture medium in the experiments. A volume of 100 µL of TSB was added to each well of the sterile 96-well microplates. The initial concentration of the postbiotic sample was prepared at 60 mg/mL and subjected to a two-fold serial dilution in the wells. The resulting postbiotic concentrations across ten wells were 60, 30, 15, 7.5, 3.75, 1.875, 0.937, 0.468, 0.234, and 0.117 mg/mL, respectively. Bacterial suspensions prepared from fresh cultures were diluted to 10^6 CFU/mL. Subsequently, 100 µL of each bacterial suspension was added to the wells, and the plates were incubated at 37°C for 24 hours. Post-incubation, bacterial growth was measured spectrophotometrically at 600 nm. The MIC value was defined as the lowest postbiotic concentration that completely inhibited visible bacterial growth compared to the control group (Patel, 2017; Yildirim et al., 2025).

For MBC determination, 20 µL was taken from the wells showing no visible growth in the MIC test and plated onto selective media specific to each bacterium. Xylose Lysine Deoxycholate Agar (XLD) was used for *Salmonella* Typhimurium, Oxford Agar for *Listeria monocytogenes*, and Eosin Methylene Blue Agar (EMB) for *Escherichia coli*. The plates were incubated at 37°C for 24 hours, and colony development was evaluated. The lowest

concentration at which no colony development was observed at the end of incubation was accepted as the MBC value. All experiments were conducted in three independent replicates.

Statistical Analysis

The statistical analysis of the data was performed using SPSS 25.0 software. Descriptive statistics were conducted to evaluate the MIC and MBC data. Results are presented as mean ± standard error.

RESULTS AND DISCUSSION

In this study, the antibacterial efficacy of whole-cell postbiotic derived from mastic-associated bacteria was assessed against three significant foodborne pathogens: *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7. The results indicated that the minimum inhibitory concentration (MIC) was consistently 15 mg/mL for all tested microorganisms. The minimum bactericidal concentration (MBC) values were determined to be 60 mg/mL for *S.* Typhimurium and *L. monocytogenes,* and 30 mg/mL for *E. coli O157:H7* (Table 1 and Figure 3). These findings suggest that the postbiotic exhibits broad-spectrum antibacterial activity, with the bactericidal effect varying according to the specific target microorganism.

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of postbiotics.

Strain	MIC (mg/mL)	MBC (mg/mL)
Salmonella Typhimurium ATCC 14028	15± 0.00	60± 0.00
Listeria monocytogenes RSKK 472	15± 0.00	60± 0.00
Escherichia coli O157:H7 ATCC 43894	15± 0.00	30± 0.00

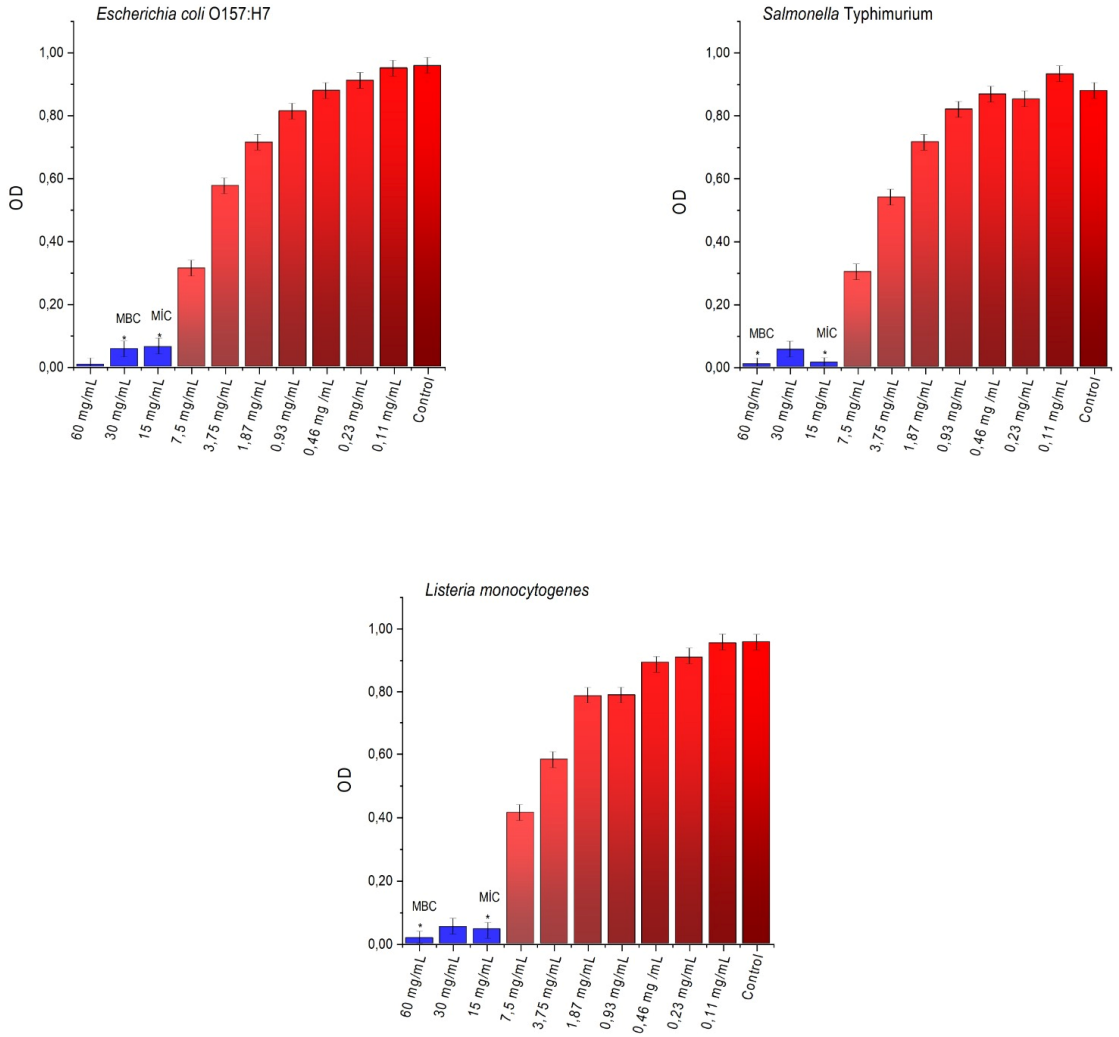

Figure 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of postbiotics.

The findings presented align with previous research indicating that postbiotics exhibit broad-spectrum antibacterial properties. Specifically, Rahman et al. (2025) reported that postbiotics derived from lactic acid bacteria are effective against both Gram-positive and Gram-negative bacteria, with notable inhibition observed against *E. coli* species. Similarly, Chang et al. (2021) demonstrated that postbiotics produced by *Lactiplantibacillus plantarum* possess strong antimicrobial activity, attributed to their organic acid content. Furthermore, Aguilar-Toalá et al. (2018) identified that the organic acids, short-chain fatty acids, and bacteriocin-like compounds present in postbiotics contribute to their broad-spectrum antimicrobial efficacy.

In this study, the observation that the MIC values for all bacterial strains were 15 mg/mL indicates that the inhibitory effect of the postbiotic is primarily attributable to common metabolites. However, the variations in MBC values can be attributed to the structural characteristics of the bacteria. Although it is generally recognized that Gram-negative bacteria exhibit greater resistance due to their outer membrane structure, it is noteworthy that in this

study, *E. coli* O157:H7 demonstrated a lower MBC value. This finding suggests that postbiotic components may exert a bactericidal effect by compromising the integrity of the cell membrane. Indeed, the antimicrobial effects of organic acids, bacteriocins, and other bioactive metabolites present in postbiotics are reported to occur through mechanisms such as reduction of intracellular pH, increased membrane permeability, and inhibition of energy metabolism (Mishra et al., 2024; Moghadam et al., 2026). Furthermore, it has been demonstrated that organic acids induce bacterial cell death by suppressing enzymatic activity in pathogenic bacteria and disrupting the intracellular proton balance (Prajapati et al., 2023). Conversely, the observation of higher MBC values for *S.* Typhimurium and *L. monocytogenes* suggests that these bacteria possess more resilient structures. This phenomenon can be particularly explained by *L. monocytogenes* ability to adapt to stress conditions and the robustness of its cell wall structure against antimicrobial agents. Studies on *L. monocytogenes* have shown that this bacterium can develop high tolerance to environmental stresses and may therefore require higher antimicrobial concentrations (Sibanda et al., 2022; Wang et al., 2023).

Fermented dairy products serve as a significant source of biologically active compounds, attributable to their intricate microbial compositions. In this regard, the demonstration of the antibacterial efficacy of postbiotics derived from traditional products such as mastik underscores the potential application of these products as natural biopreservatives. Indeed, research within the domain of food microbiology has indicated that postbiotics derived from lactic acid bacteria (LAB) are effective in controlling pathogens within food systems (Jalali et al., 2024; Serter et al., 2024; Tong et al., 20225). Moreover, the findings of İncili et al. (2023) corroborate this study by demonstrating that whole-cell postbiotics significantly inhibited pathogen growth in model food systems.

CONCLUSION

In this study, it was demonstrated that whole-cell postbiotics derived from bacteria isolated from mastic, a traditional fermented dairy product, exhibited a significant antibacterial effect against major foodborne pathogens, namely *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7. The obtained MIC and MBC values indicate that the postbiotic in question is effective against both Gram-positive and Gram-negative bacteria, and it can exhibit bactericidal effects at lower concentrations, particularly in some pathogens. The findings suggest that traditional fermented products like mastic have significant potential as natural bioprotective agents. However, it is recommended that the composition of the postbiotic be thoroughly characterized, its mechanisms of action clarified, and its applicability in different food systems investigated.

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