KSÜ Tarım ve Doğa Derg 28 (6), 1405-1413, 2025
KSU J. Agric Nat 28 (6), 1405-1413, 2025
https://doi.org/10.18016/ksutarimdoga.vi.1701368

Modeling the Effect of Pediocin Application on the Growth and Survival of *Listeria monocytogenes*

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ABSTRACT
The aim of this study was to investigate the effect of pediocin on *Listeria monocytogenes* under optimal temperature conditions and to model the bacterial growth and survival dynamics in the presence and absence of pediocin. The susceptibility of 28 *L. monocytogenes* strains to pediocin was evaluated using Minimum Inhibitory Concentration (MIC) tests. To determine the antimicrobial efficacy of pediocin, solutions at concentrations of 7 µg/mL and 12 µg/mL were applied to the strains, including the reference strain, and the inhibitory effect on bacterial growth was assessed at 35 °C. Model parameters were estimated using R-BioXL software, with model performance supported by R^2, adjusted R^2, and RMSE values. The results showed that the *L. monocytogenes* 287-1P strain exhibited higher resistance to pediocin compared to other strains. Although pediocin reduced the growth rate and delayed bacterial proliferation, its effect as a sole treatment was limited. Therefore, it is recommended that pediocin be used in combination with other preservative strategies within a hurdle technology framework. The findings indicate that pediocin has potential as a bioprotective agent for controlling *L. monocytogenes* in food products, contributing to enhanced microbial safety and public health protection. Furthermore, modeling the effect of pediocin provides a valuable tool for evaluating the growth and survival dynamics of this pathogen.

Microbiology

Research Article

Article History
Received : 17.05.2025
Accepted : 26.07.2025

Keywords
Listeria monocytogenes
Biocontrol
Pediocin
Modelling

Pediyosin Uygulamasının *Listeria monocytogenes*'in Büyüme ve Yaşam Sürecine Etkisinin Modellenmesi

ÖZET
Bu çalışmanın amacı, pediyosinin *Listeria monocytogenes* üzerindeki etkisini optimum sıcaklık koşullarında incelemek ve pediyosin varlığında ya da yokluğunda bakterinin büyüme ve hayatta kalma süreçlerini modellemektir. Çalışmada, 28 farklı *L. monocytogenes* suşunun pediyosine duyarlılığı Minimum İnhibitör Konsantrasyon (MIK) testleri ile değerlendirilmiştir. Pediyosinin antimikrobiyal etkisini belirlemek için suşlara 7 µg/mL ve 12 µg/mL konsantrasyonlarında pediyosin uygulanmış, 35 °C'de bakteriyel büyüme üzerindeki inhibitör etkisi incelenmiştir. Model parametreleri, R-BioXL yazılımı kullanılarak tahmin edilmiş ve model performansı R^2, düzeltilmiş R^2 ($R^2_a d_i$) ve RMSE değerleri ile desteklenmiştir. Sonuçlar, *L. monocytogenes* 287-1P suşunun pediyosin direncinin diğer suşlara göre daha yüksek olduğunu göstermiştir. Pediyosin, büyüme hızını düşürerek patojenin çoğalmasını geciktirse de, tek başına uygulandığında sınırlı bir kontrol sağlamaktadır. Bu nedenle, pediyosinin diğer koruyucu yöntemlerle birlikte, "hurdle teknolojisi" kapsamında kullanılması önerilmektedir. Elde edilen bulgular, pediyosinin gıda ürünlerinde *L. monocytogenes* kontrolünde biyokoruyucu ajan olarak potansiyel taşıdığını ve bu sayede mikrobiyal güvenliğin artırılarak halk sağlığının korunmasına katkı sağlanabileceğini ortaya koymaktadır. Ayrıca, pediyosin etkisinin

Mikrobiyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 17.05.2025
Kabul Tarihi : 26.07.2025

Anahtar Kelimeler
Listeria monocytogenes
Biyokontrol
Pediyosin
Modelleme

modellenmesi, patojenin büyüme ve hayatta kalma dinamiklerinin değerlendirilmesinde etkili bir araç olarak önem taşımaktadır.

Atıf Şekli: Toprak, Z.T., Şanlıbaba, P., & Buzrul, S. (2025). Pediyosin Uygulamasının *Listeria monocytogenes*'in Büyüme ve Yaşam Sürecine Etkisinin Modellenmesi. *KSÜ Tarım ve Doğa Derg 28* (6), 1405-1413. https://doi.org/10.18016/ksutarimdoga.vi.1701368

To Cite : Toprak, Z.T., Şanlıbaba, P., & Buzrul, S. (2025). Modeling the Effect of Pediocin Application on the Growth and Survival of *Listeria monocytogenes. KSU J. Agric Nat 28* (6), 1405-1413. https://doi.org/10.18016/ksutarimdoga.vi.1701368

INTRODUCTION

Listeria monocytogenes is a Gram-positive, nonsporulating, rod-shaped, aerobic or facultative anaerobic bacterium responsible for the foodborne illness known as listeriosis (Gray et al., 2018; Thakur et al., 2018). Listeriosis primarily impacts vulnerable populations, including young children, pregnant women, the elderly, and individuals with compromised immune systems. This is further substantiated by epidemiological data indicating that the majority of reported foodborne listeriosis cases occur in individuals over the age of 64 (Çolak et al., 2008; Morandi et al., 2019). In healthy individuals, listeriosis typically presents as a mild, self-limiting illness characterized by influenza-like symptoms and gastroenteritis. However, in high-risk populations, the infection can lead to severe complications, such as septicemia, meningitis, and adverse pregnancy outcomes, including miscarriage and stillbirth (Grigore-Gurgu et al., 2024). Although *Listeria monocytogenes*-associated illness is less common than infections caused by other foodborne pathogens such as *Salmonella, Campylobacter,* or *Escherichia coli*, it is associated with the highest mortality rate among them. Approximately 30% of invasive listeriosis cases result in fatal outcomes, with the majority requiring hospitalization. Due to its severe clinical manifestations and high fatality rate, *L. monocytogenes* is recognized as a foodborne pathogen of significant public health concern (Gray et al., 2018; EFSA, 2023).

Currently, biological control strategies are being employed to prevent and mitigate the growth of *L. monocytogenes* in food. These approaches include the use of bacteriophages, competitive bacterial species such as lactic acid bacteria (LAB), bacteriocins, essential oils, as well as endolysins and their derivatives (Połaska & Sokołowska, 2019; Liu et al., 2022). These biocontrol agents offer promising alternatives to conventional antimicrobial treatments, enhancing food safety while minimizing the risk of antimicrobial resistance (Grigore-Gurgu et al., 2024). The majority of bacteria produce at least one bacteriocin, with LAB being well-known for their prolific bacteriocin production (Lahiri et al., 2022; Maldonado-Barragán et al., 2022). Bacteriocins are ribosomally synthesized antimicrobial peptides, typically ranging from 20 to 60 amino acids in length. These peptides are cationic and hydrophobic, enabling them to effectively inhibit the growth of both Gram-negative and Gram-positive food spoilage and pathogenic bacteria. Due to their broad-spectrum antimicrobial activity, bacteriocins represent a promising strategy for combating foodborne pathogens and enhancing food safety (Kumariya et al., 2019; Fidan et al., 2022). Bacteriocins have been broadly classified into four general classes based on their composition and structural properties (Lahiri et al., 2022). The first class, known as lantibiotics, consists of bacteriocins that contain unusual amino acids, such as lanthionines and ß-methyllanthionines. The biosynthesis of lantibiotics involves post-translational modifications, which have been extensively studied in the case of nisin. The second class comprises bacteriocins that lack lanthionine residues and are characterized by their heat stability and mechanism of action, primarily targeting the cell membrane. The third class consists of large, heat-sensitive bacteriocins, while the fourth class includes bacteriocins that incorporate additional chemical moieties, such as carbohydrates and lipids (Martín et al., 2022).

Among the bacteriocins produced by LAB, nisin is the most extensively studied lantibiotic for the biocontrol of *L. monocytogenes* in food systems. Its well-documented antimicrobial efficacy, stability, and regulatory approval for food applications have made it a key biopreservative in the food industry (O'Connor et al., 2020). In addition to the well-characterized lantibiotic nisin, Class II bacteriocins, particularly those belonging to subclass IIa, exhibit strong antimicrobial activity against *L. monocytogenes*. Several notable bacteriocins in this category include garviecin LG34, bifidocin A, leucocin C-607, pediocin GS4, plantaricin LPL-1, pediocin PA-1, and sakacins. These bacteriocins are of significant interest due to their potent antilisterial activity, making them valuable candidates for biopreservation and food safety applications (Martín et al., 2022). Pediocin, a member of subclass IIa, is produced by various *Pediococcus* species, including *Pediococcus acidilactici*, *P. claussenii*, *P. cellicola*, *P. damnosus*, *P. ethanolidurans*, *P. inopinatus*, *P. parvulus*, *P. pentosaceus*, and *P. stilesii* (Balandin et al., 2019). Depending on the isomer and the specific producing strain, bacteriocins produced by these species are often given distinguishing symbols, such as AcH, SJ-, JD, and PA-1. Due to its proteinaceous nature, pediocin exerts its inhibitory effect primarily through pore formation in the target cell membrane, leading to cell death. Its notable thermal stability allows it to retain activity even after exposure to high temperatures, making it suitable for various food processing

applications. Pediocin has been widely used as a natural biopreservative in the food industry, especially in ready-to-eat meat products, dairy, and fermented foods, to improve microbial safety and extend shelf life. Its ability to inhibit foodborne pathogens while being generally recognized as safe (GRAS) has positioned pediocin as a promising alternative to chemical preservatives. It is known for its ability to tolerate low temperatures and maintain its antimicrobial activity across a broad pH range. Numerous studies have highlighted pediocin's effectiveness against *L. monocytogenes*, demonstrating its ability to reduce *L. monocytogenes* populations in various food products. This makes pediocin a promising biopreservative for enhancing food safety, particularly in controlling listeriosis (Khorshidian et al., 2021). The MicroGARD™ range and ALTA 2431, both containing pediocin PA-1, which have been approved by the FDA as bacteriocin-containing fermentates (Espitia et al., 2016; Liu et al., 2022). Although pediocin has been approved for use as a food additive due to its antimicrobial efficacy, limited attention has been given to consumer perception and its potential allergenicity. It is important to consider that despite regulatory approval, consumer acceptance may be influenced by factors such as naturalness, labeling, and familiarity with the additive. Moreover, while current studies suggest that pediocin is generally regarded as safe (GRAS), comprehensive assessments regarding its allergenic potential, especially in sensitive individuals, remain scarce (Khorshidian et al.,2021). Future studies should address these aspects to ensure both the safety and acceptability of pediocin as a biopreservative in the food industry.

Mathematical modeling provides a valuable framework for predicting the behavior of foodborne pathogens under various environmental conditions and antimicrobial treatments. In this study, modeling was used to quantitatively assess the effects of pediocin on the growth kinetics of *Listeria monocytogenes*. Predictive models not only facilitate the understanding of microbial responses but also offer practical tools for food safety risk assessment and the development of preservation strategies. Previous research has highlighted the relevance of such approaches in food microbiology. McKellar and Lu (2004) demonstrated the general applicability of microbial response modeling in food systems, while Mejlholm and Dalgaard (2009) predicted the growth limits of *L. monocytogenes* in seafood treated with lactic acid bacteria and bacteriocins. Specifically related to pediocin, for instance, Jagannath et al. (2001) developed polynomial predictive equations to simulate the survival of *L. monocytogenes* Scott A in Shrikhand—a traditional dairy product—under varying pediocin K7 levels, storage temperatures, inoculum sizes, and durations. More recently, modeling studies have applied response surface methodologies to assess the combined effects of pediocin, sodium lactate, sodium diacetate, and thermal treatments on *L. monocytogenes* in bologna, reporting significant interactions that influence D-values under different temperature and additive conditions (Maks et al., 2010). These studies underscore that pediocin's antimicrobial efficacy cannot be separated from environmental context and formulation variables, and they demonstrate the value of modeling to capture such complexity. In line with these findings, our modeling approach quantitatively simulates the dose- and temperature-dependent effects of pediocin on both maximum cell counts and growth rate, revealing its limited standalone efficacy and reinforcing the need for its integration into hurdle technology-based interventions for improved microbial control.

The aim of our study was to describe the growth and subsequent survival of *L. monocytogenes with or without the addition of pediocin by using a model with interpretable parameters.* We wanted to monitor the effect of pediocin on *Listeria* at the optimum temperature.

MATERIAL and METHOD

Bacteria and Growth Conditions

Previously isolated from ready-to-eat foods in Ankara/Türkiye and characterized 28 *L. monocytogenes* strains (Şanlıbaba et al., 2018) and *L. monocytogenes* ATCC7644 were used in this study. The selection of 28 strains in this study was carried out with the aim of covering a broad spectrum of species and subspecies that are frequently encountered in the food industry. The *Listeria* strains were sourced from the culture collection of the Food Microbiology Laboratory, Department of Food Engineering, Ankara University, Ankara, Türkiye. Each strain was individually cultured in Tryptic Soy Broth supplemented with 0.6% yeast extract (TSBYE) (Sigma, Germany) at 35 °C for 20–24 h. The *Listeria* species strains were preserved at −20°C with 30% (v/v) glycerol (Merck, Germany).

Preparation of Pediocin Solution and Determination of Minimum Inhibitory Concentration (MIC)

Pediocin stock solutions (Pediocin PA-1, Catalog Number P0098, 133108-87-9, Sigma Aldrich) were supplied as a solution (0.1 mg/mL in 0.1 M sodium acetate, pH 5.0). The pediocin solutions were stored at 4°C. The minimum inhibitory concentration (MIC) assay was employed to assess the antimicrobial activity of pediocin against *L. monocytogenes* strains. MIC determinations were conducted in triplicate using 96-well microtiter plates, following the methodology outlined by Lynch et al. (2021).

Determination of the Sensitivity of *Listeria monocytogenes* Strains to Pediocin

The antimicrobial activity of pediocin against *L. monocytogenes* strains and a reference strain was evaluated at 35 °C. Initially, bacterial cultures were prepared in sterile Tryptic Soy Broth (TSB) medium for 18 h at a cell density of 10^5 CFU/mL. Subsequently, pediocin solutions at two concentrations (7 µg/mL and 12 µg/mL) were added into each cell density culture at a volume of 100 µL. Each experimental tube containing pediocin and *L. monocytogenes* was then incubated at 35°C. During the storage period, samples were taken at regular intervals to perform both viable cell counting and to measure the optical density (OD) at 625 nm using a spectrophotometer (Khanipour et al., 2016). Uninoculated samples served as negative controls, while TSBYE without pediocin, inoculated with *Listeria monocytogenes* at initial concentrations of 10^5 CFU/mL, functioned as positive controls.

Modeling

The following model with four parameters was used to describe the growth and death of *L. monocytogenes* with or without pediocin: where $\log_{10}N(t)$ is the number of cells at a time t, $\log_{10}N_{max}$ is the maximum number of cells which can be reached at t_{max}, μ and k are the growth rate and death rate (time^{-1}), respectively.

$$\text{If } t \le t_{max};\ \log_{10} N(t) = \log_{10} N_{max} + \frac{\mu}{\ln 10}(t - t_{max}) \quad (1.1)$$

$$\text{If } t > t_{max};\ \log_{10} N(t) = \log_{10} N_{max} - \frac{k}{\ln 10}(t - t_{max}) \quad (1.2)$$

These parameters are shown in Figure 1 together with a hypothetical model.

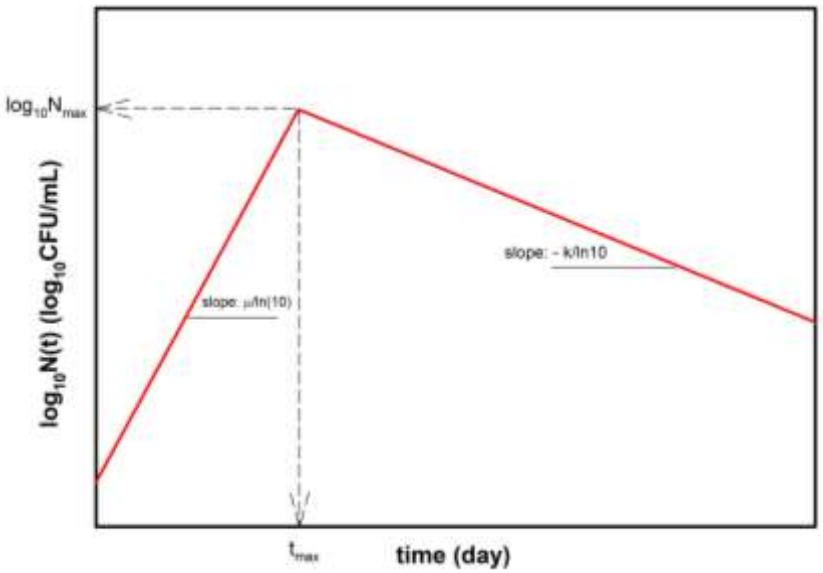

Figure 1. Illustration of Eq.(1) (red lines) and parameters of the model ($\log_{10}N_{max}$, t_{max}, µ and k)
Şekil 1. Denklem (1)'in şekil üzerinde gösterimi (kırmızı çizgiler) ve model parametreleri ($log_{10}Nmax$, tmax, µ ve k)

Parameters of the model [Eq.(1)] were obtained by R-BioXL (Öksüz & Buzrul, 2024) together with their standard errors and symmetric confidence intervals (calculated by linear approximation). Determination coefficient (R^2), adjusted determination coefficient (R^2_{adj}), and root mean square error (RMSE) values were also listed by R-BioXL.

RESULTS and DISCUSSION

Several studies examined the use of pediocin-producing strains as biocontrol agents against *L. monocytogenes* strains (Chen et al., 2004; Huang et al., 2009; Bahrami et al., 2020; Komora et al., 2020; Ramos et al., 2020). To the best of our knowledge, our study is the first one using pediocin powder preparation against *L. monocytogenes* strains. *L. monocytogenes* is an intracellular pathogen capable of proliferating over a wide temperature range, from −0.4°C to 50°C, with an optimal growth temperature between 30°C and 37°C. This remarkable adaptability allows *L. monocytogenes* to persist in various environments, including refrigerated food products, posing a significant challenge to food safety (Thakur et al., 2018). For this reason, the optimal growth temperature of *L. monocytogenes*, 35°C, was used in this study.

Twenty-eight *L. monocytogenes* strains were tested for susceptibility to pediocin using MIC assays. The results demonstrated inhibitory effects of pediocin against all tested strains (data not given). Among the selected strains, 287-1P exhibited the highest pediocin resistance. In contrast, *L. monocytogenes* ATCC 7644 was highly sensitive

to pediocin. Figure 2 shows the effect of pediocin on *L. monocytogenes* ATCC7644, and Table 1 lists the model parameters.

Table 1. Parameters and their 95% confidence intervals (given in parentheses) and goodness-of-fit of Eq.(1) for L. monocytogenes ATCC7644

Çizelge 1. L. monocytogenes ATCC7644 suşu için Denklem (1)'in parametreleri ve %95 güven aralıkları (parantez içinde verilmiştir) ile modelin uyum iyiliği (goodness-of-fit) değerleri

	$\log_{10}N_{max}$ ($\log_{10}$CFU/mL)	t_{max} (day)	μ (day^{-1})	k (day^{-1})	R^2	R^2_{adj}	RMSE
Control	10.05 (9.68-10.42)	3.94 (3.46-4.42)	4.20 (3.11-5.30)	0.94 (0.77-1.11)	0.9713	0.9617	0.2973
7 µg/mL	9.68 (9.44-9.92)	3.90 (3.56-4.24)	4.04 (3.33-4.75)	0.65 (0.54-0.76)	0.9854	0.9805	0.1920
Control	9.68 (9.20-10.16)	2.37 (2.00-2.73)	8.40 (5.26-11.54)	0.44 (0.32-0.56)	0.9410	0.9213	0.4263
12 µg/mL	9.14 (8.74-9.54)	2.34 (2.01-2.66)	7.67 (5.08-10.26)	0.29 (0.19-0.39)	0.9436	0.9248	0.3519

In general, our model produced good fits as can be judged by visual examination (Figure 2) as well as goodness-of-fit indicators (Table 1). The nature of the data (fast growth followed by slow inactivation) made the use of alternative models difficult because such data can be described by at least four parameters. We decided to use Eq.(1) due to the rule of parsimony i.e., model with minimum number of parameters is the best model.

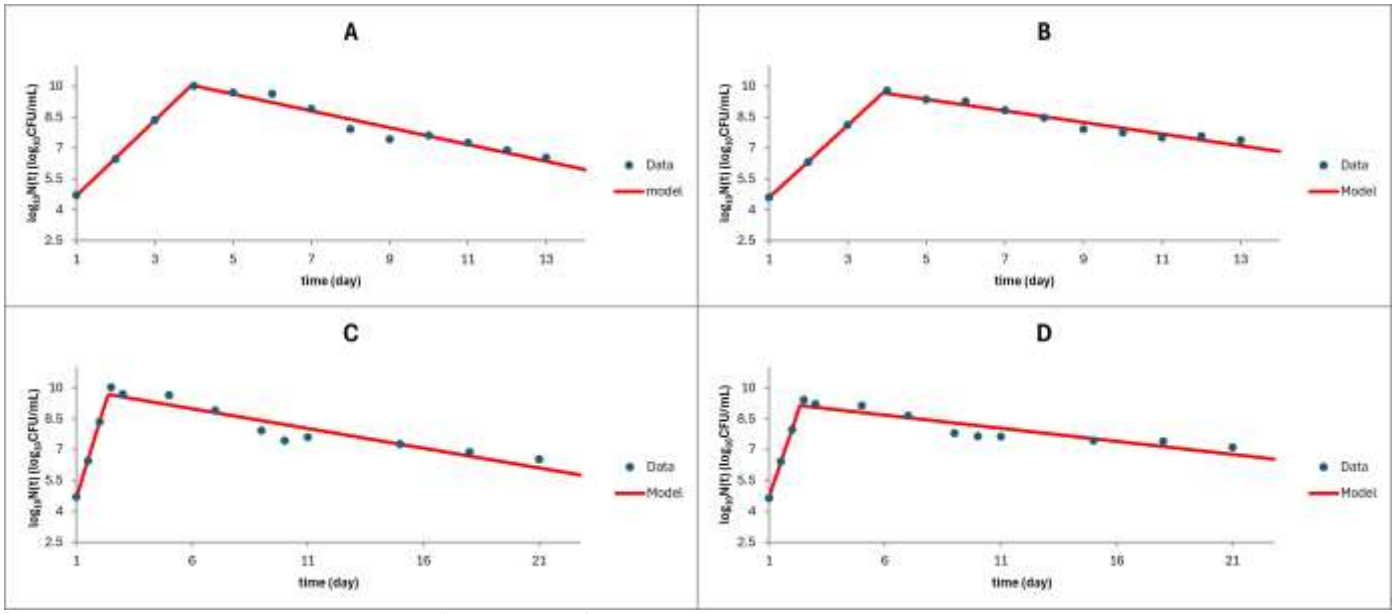

Figure 2. Growth and death data (blue circles) of L. monocytogenes ATCC7644 and fit of the proposed model [Eq.(1)] (red lines). Control (no pediocin) sample (A) and its 7 µg/mL pediocin applied counterpart (B), control (no pediocin) sample (C) and its 12 µg/mL pediocin applied counterpart (D)

Şekil 2. L. monocytogenes ATCC7644 suşuna ait büyüme ve ölüm verileri (mavi daireler) ile önerilen modelin [Denklem (1)] uyumu (kırmızı çizgiler). Pediocin içermeyen kontrol örneği (A) ve buna karşılık gelen 7 µg/mL pediocin uygulanmış örnek (B); pediocin içermeyen diğer bir kontrol örneği (C) ve buna karşılık gelen 12 µg/mL pediocin uygulanmış örnek (D).

Pediocin slightly reduced $\log_{10}N_{max}$ (0.37 and 0.54 $\log_{10}$CFU/mL reductions for 7 and 12 µg/mL pediocin applications, respectively, and these correspond to decreases of 3.7% and 5.6%, respectively). It also had an impact on μ (0.16 and 0.73 day^{-1} reductions for 7 and 12 µg/mL pediocin applications, respectively, and these correspond to decreases of 3.8% and 8.7%, respectively) compared to control samples, indicating that pediocin added samples had slower growth. Although the growth was slower, the effect of pediocin on growth rate (μ) is statistically insignificant ($p > 0.05$) since the 95% confidence intervals overlapped (Table 1). On the other hand, it had little effect on t_{max} i.e., cells almost reached their maximum value at the same time with or without pediocin. Interestingly, k values were higher for control samples (Table 1).

Figure 3 shows the effect of pediocin on *L. monocytogenes* 287-1P and Table 2 lists the model parameters.

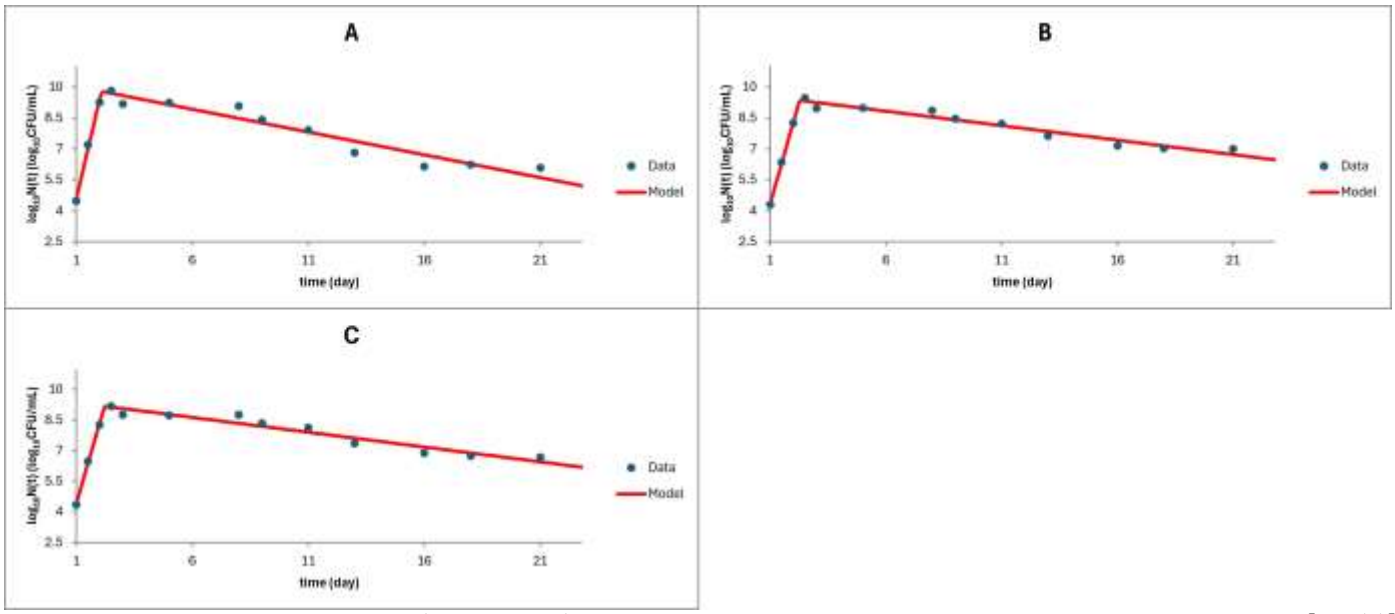

Figure 3. Growth and death data (blue circles) of L. monocytogenes 287-1P and fit of the proposed model [Eq.(1)] (red lines). Control (no pediocin) sample (A) and its 7 µg/mL pediocin (B), 12 µg/mL pediocin (C) applied counterparts.

Şekil 3. L. monocytogenes 287-1P suşuna ait büyüme ve ölüm verileri (mavi daireler) ile önerilen modelin [Denklem (1)] uyumu (kırmızı çizgiler). Pediocin içermeyen kontrol örneği (A) ve buna karşılık gelen 7 µg/mL pediocin uygulanmış örnek (B) ile 12 µg/mL pediocin uygulanmış örnek (C).

Table 2. Parameters and their 95% confidence intervals (given in parentheses) and goodness-of-fit of Eq.(1) for L. monocytogenes 287-1P

Çizelge 2. L. monocytogenes 287-1P suşu için Denklem (1)'in parametreleri, %95 güven aralıkları (parantez içinde verilmiştir) ve modelin uyum iyiliği (goodness-of-fit) değerleri.

	$\log_{10}N_{max}$ ($\log_{10}$CFU/mL)	t_{max} (day)	μ (day^{-1})	k (day^{-1})	R^2	R^2_{adj}	RMSE
Control	9.78 (9.29-10.26)	2.09 (1.88-2.30)	10.98 (7.99-13.98)	0.51 (0.40-0.62)	0.9542	0.9389	0.4063
7 µg/mL	9.35 (9.09-9.61)	2.27 (2.11-2.43)	9.10 (7.48-10.71)	0.32 (0.26-0.38)	0.9816	0.9754	0.2190
12 µg/mL	9.18 (8.87-9.48)	2.22 (2.03-2.40)	9.03 (7.15-10.90)	0.33 (0.26-0.40)	0.9732	0.9642	0.2543

Similar to *L. monocytogenes* ATCC7644 strain, slight decreases in $\log_{10}N_{max}$ (0.35 and 0.53 $\log_{10}$CFU/mL reductions for 7 and 12 µg/mL pediocin applications, respectively, and these correspond to decreases of 4.4% and 6.1%, respectively) were found compared to the control sample. Pediocin added samples had 0.18 days (> 4 h) and 0.13 days (> 3 h) higher t_{max} values, respectively. On the other hand, strain 287-1P had more pronounced decreases in μ (1.88 and 1.95 day^{-1} reductions for 7 and 12 µg/mL pediocin applications, respectively, and these correspond to decreases of 17.1% and 17.8%, respectively) than that of the ATCC strain. The effect of pediocin was statistically significant ($p \leq 0.05$) on growth rate (μ) for this strain. Once again, k value of the control sample was higher than the pediocin added samples (Table 2).

Normally, a decrease in the number of cells is expected in samples treated with pediocin. For example, Kiran and Osmanagaoglu (2014) reported a significant reduction of 3.8 $\log_{10}$ CFU/g in *L. monocytogenes* populations in raw chicken meat after two weeks at 4 °C; however, regrowth occurred after prolonged storage (3–4 weeks). A slight reduction (~0.5 $\log_{10}$) was observed at 24 h (data not shown), indicating an initial inhibitory effect of pediocin in this study. Nevertheless, due to incubation at 35 °C, growth eventually resumed both in the presence and absence of pediocin (Figure 2 and Figure 3). Importantly, the growth rate was significantly slower in pediocin-treated samples (Table 1 and Table 2), demonstrating pediocin's inhibitory influence under the tested conditions. Pediocin can be used to delay the growth of *L. monocytogenes* even at a high temperature, as it reduced the growth rate (μ) but its usage alone is not very effective in this study. Therefore, the hurdle concept can be an alternative for this pathogen. Nevertheless, it should be noted that pediocin applications were done at the optimum temperature (35 °C), and a lower temperature might have a different impact on the growth. Chen et al. (2004) used pediocin to inhibit the growth of *L. monocytogenes* in frankfurters at 4, 10, and 25 °C and observed that pediocin

application at 4 °C effectively reduced the growth of *L. monocytogenes* up to 12 weeks. Moreover, the maximum cell number reached during growth can be reduced with the addition of pediocin. Pediocin slowed down the death rate (*k*) of *Listeria* after the growth phase (Figure 2 and Figure 3); however, if pediocin and a lethal treatment such as heat or pressure are used together, it would increase the inactivation rate.

While pediocin exhibits strong antimicrobial activity under controlled laboratory conditions, its stability and efficacy can be significantly influenced by the complexity of food matrices. Factors such as pH, temperature, enzymatic activity, and interactions with food components (e.g., proteins and fats) may contribute to its degradation or reduced bioactivity in real food systems. Studies have shown that pediocin can be inactivated or adsorbed by food components, leading to a diminished inhibitory effect against target pathogens (Rodríguez et al., 2002). Additionally, heat treatments commonly applied during food processing may partially denature pediocin, further limiting its effectiveness (Chen et al., 2004). To address these limitations, various combination strategies have been investigated to enhance the efficacy of pediocin in food systems. For instance, the use of pediocin in conjunction with high hydrostatic pressure (HHP) has shown synergistic effects. Bruschi et al. (2017) investigated the effect of high hydrostatic pressure (HHP: 300, 400, and 500 MPa at 10 °C for 5 min) together with pediocin on the survival of 14 strains of *L. monocytogenes*. They found that the pediocin had an antibacterial activity against all strains, and exposure to HHP resulted in an increase in bacterial susceptibility to pediocin. However, it should be noted that although the combination of pediocin with HHP is promising, the high cost associated with pressure-based technologies—including both equipment and operational expenses—makes it less feasible than conventional thermal treatments. Therefore, combining pediocin with mild heat treatments may represent a more practical and cost-effective hurdle technology for ensuring microbial safety while maintaining product quality (Zacharof & Lovitt, 2012). From an industrial perspective, hurdle technology offers a practical and flexible approach to food preservation by combining multiple mild preservation methods to achieve microbial safety without compromising product quality. In real-world applications, the integration of bacteriocins like pediocin with sublethal heat treatment, mild acidification, or packaging technologies has proven effective in extending shelf life while maintaining sensory and nutritional properties. This multi-targeted strategy reduces reliance on harsh processing or synthetic additives, aligning with consumer demand for minimally processed, 'clean label' foods. As such, hurdle technology is increasingly being adopted in meat, dairy, and ready-to-eat food sectors for its adaptability, cost-effectiveness, and regulatory compliance (Khan et al., 2017).

The observed decrease in the death rates of *Listeria monocytogenes* following pediocin treatment may be attributed to the mode of action of pediocin, a class IIa bacteriocin known for its anti-listerial activity. Pediocin primarily exerts a bactericidal effect by forming pores in the cytoplasmic membrane, leading to leakage of intracellular contents and cell death. However, under certain environmental conditions or at sublethal concentrations, its action may shift toward a bacteriostatic effect, thereby inhibiting cell division without causing immediate cell death. This may result in a lower apparent death rate in the bacterial population. Furthermore, exposure to bacteriocins like pediocin may induce a stress response in some *Listeria* cells, allowing them to enter a transient viable-but-non-culturable (VBNC) state or activate stress resistance mechanisms, which may also contribute to the reduced death rate observed in this study.

CONCLUSION

In this study, the effects of pediocin application on the growth and survival dynamics of *L. monocytogenes* at 35 °C were evaluated using an appropriate mathematical modelling approach. Pediocin has gained significant interest for industrial applications, particularly in the food industry, due to its ability to inhibit *L. monocytogenes* and other foodborne pathogens, thereby enhancing the microbiological safety and shelf life of ready-to-eat products. Pediocin treatment significantly reduced the bacterial growth rate and maximum population density. However, no notable change was observed in the time required to reach the maximum population density. Interestingly, samples treated with pediocin exhibited a lower death rate after the growth phase, i.e., once the maximum population density was reached, compared to control samples. This finding suggests a complex effect of pediocin on the surviving bacterial populations and indicates the need for more detailed mechanistic studies. Overall, mathematical modeling proved to be a powerful and useful tool for understanding the antimicrobial effects of pediocin on *L. monocytogenes*. Future research is recommended to further investigate these differential effects of pediocin and the role of application conditions in detail.

Contribution Rate Statement Summary of Research

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors express no conflict of interest associated with this work

Funding

The Ankara University Scientific Research Projects Coordination Unit (Project number 21H0443002) supported this work.

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