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Use of Silver Nanoparticles in the Inhibition of *Listeria spp.*

Utilização de Nanopartículas de Prata na Inibição de *Listeria* spp.

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Abstract
Fresh meat and meat products can become vehicles for the spread of pathogens such as *Listeria* spp. The use of antimicrobials during food processing is an effective strategy to control microbial growth. In this context, nanotechnology, especially the application of silver nanoparticles (NPAg), stands out as a promising alternative for the food industry. The present work aimed to use silver nanoparticles in the control of Listeria spp. The strains studied were *Listeria monocytogenes*, *Listeria innocua* and *Listeria ivanovii* and the concentrations of the colloidal silver solution were 2, 3, 4, 5, 10 and 20 mg/L. The minimum inhibitory concentration in microplates and the measurement of the minimum bactericidal concentration were evaluated to verify the inhibition through the presence or absence of growth. In the evaluation of antimicrobial activity, the three strains were equally inhibited, demonstrating sensitivity from a concentration of 10 mg/L of silver. In determining the minimum bactericidal concentration, *L. monocytogenes* demonstrated greater sensitivity, with inhibition from 5

mg/L. Silver nanoparticles present antimicrobial action against *Listeria* spp.

Keywords: Pathogen. Food Security. Antimicrobial. Biotechnology.

Resumo
Carnes *in natura* e produtos cárneos podem se tornar veículos para a disseminação de patógenos como *Listeria* spp. O uso de antimicrobianos durante o processamento de alimentos é uma estratégia eficaz para controlar o crescimento microbiano. Nesse contexto, a nanotecnologia, especialmente a aplicação de nanopartículas de prata (NPAg), destaca-se como uma alternativa promissora para a indústria alimentícia. O presente trabalho teve como objetivo a utilização de nanopartículas de prata no controle de *Listeria* spp. As estirpes estudas foram *Listeria monocytogenes, Listeria innocua e Listeria ivanovii* e as concentrações da solução de prata coloidal foram de 2, 3, 4, 5, 10 e 20 mg/L. A concentração inibitória mínima em microplacas e a medida da concentração bactericida mínima foram avaliadas para verificar a inibição por meio de presença ou ausência de crescimento. Na avaliação da atividade antimicrobiana, as três estirpes foram igualmente inibidas, demonstrando sensibilidade a partir da concentração de 10 mg/L de prata. Na determinação da concentração mínima bactericida, a *L. monocytogenes* demonstrou maior sensibilidade, com inibição a partir de 5 mg/L. As nanopartículas de prata apresentam ação antimicrobiana contra as estirpes de *Listeria* spp.

Palavras-chave: Patógeno. Segurança Alimentar. Antimicrobiano. Biotecnologia.

1 Introduction

Microbiological safety in the food industry, particularly in meat products, significantly depends on the control of *Listeria monocytogenes*. This pathogen stands out due to its ability to survive and proliferate at refrigeration temperatures, posing a challenge for food preservation. This resistance to low temperatures is associated with the bacterium's ability to modify the lipid composition of its cell membrane, allowing it to maintain fluidity and functionality even under adverse conditions. Additionally, *L. monocytogenes* can form biofilms on industrial surfaces, facilitating its persistence in food processing environments and increasing the risk of cross-contamination (Thakur; Asrani; Patial, 2018).

Therefore, effective sanitation and monitoring strategies are essential to control the presence of this microorganism in industrial settings (Souza *et al*., 2021; Tondo; Bartz, 2019). Concern regarding this microorganism in industrial settings is of global interest. Cufaoglu, Ambarcioglu, and Ayaz (2021) observed a high prevalence of *L. monocytogenes* and *Listeria* spp., mainly associated with meat matrices and meat products in Turkey. Smith *et al*. (2019) also reported that one of the largest listeriosis outbreaks ever recorded was linked to processed meat products from January 2017 to July 2018 in South Africa, with a total of 1,060 cases and a 27% mortality rate.

Tadielo *et al*. (2023) detected the presence of *L. monocytogenes* on food conveyor belts in poultry slaughterhouses, even after undergoing pre-operational cleaning processes.

Although not pathogenic to humans, *Listeria ivanovii* is widely recognized as pathogenic to cattle and other animals (Silva *et al*., 2018; Skandamis, 2022).*Listeria innocua*, found in the intestinal

tract of warm-blooded animals (Abaya *et al*., 2019), shares the same ecological niche with *L. monocytogenes*. Therefore, the presence of *L. innocua* is indicative of *L. monocytogenes* contamination (Ahimed *et al*., 2022).

Nanotechnology has significant importance in the food industry due to its broad applicability (Rahman *et al*., 2020), enhancing flavors, textures, quality, shelf life, and food safety (Deng *et al*., 2021). Within nanotechnology, the use of silver nanoparticles (AgNPs) can serve as an alternative to traditional antimicrobials, as their small size and unique physicochemical properties enhance antimicrobial activity against major food industry pathogens (Khurshee *et al*., 2023).

Given the high relevance of *Listeria* spp. in industrial environments and the constant need to develop new antimicrobials effective against this microorganism, this study aimed to evaluate the antimicrobial potential of different concentrations of colloidal silver against three strains: *Listeria monocytogenes*, *Listeria innocua*, and *Listeria ivanovii*.

2 Material and Methods

2.1 Activation of *Listeria* spp. Strains

The antimicrobial effect of colloidal silver was evaluated on three strains of *Listeria*: *L. monocytogenes* ATCC 19111, *L. innocua* ATCC 33090, and *L. ivanovii* subsp. *londoniensis* ATCC BAA-139.

The activation of the strains was performed by transferring 10 µL of the previously thawed inoculum using a disposable loop into Brain Heart Infusion (BHI) broth (Merck), followed by incubation at 37 °C for 24 hours. After this period, each strain was streaked onto plates containing Tryptic Soy Agar (TSA, Merck) using a disposable loop and incubated at 37 °C for 48 hours.

To assess the purity of the inoculum, Gram staining was performed. Three to five colonies were collected for incubation in BHI broth and for testing with colloidal silver.

2.2 Preparation of Test NPAg Solutions

Solutions with Different Concentrations of Colloidal Silver (10, 15, 20, 25, 50, and 100%) were prepared using Demi Fraser Broth (Merck) as the solvent. These solutions were derived from a silver solution previously characterized by Valente (2021), with an initial concentration of 20 ± 2 mg/L of silver nanoparticles.

2.3 Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of colloidal silver at 10, 15, 20, 25, 50, and 100% was determined using the microdilution technique in plates, as defined by the *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* (CLSI, 2015; Duffy *et al*.,

2018). The MIC tests were conducted on *L. monocytogenes* ATCC 19111, *L. innocua* ATCC 33090, and *L. ivanovii* subsp. *londoniensis* ATCC BAA-139.

Solutions with different NPAg concentrations were prepared in duplicate, as described in section 2.2, and inoculated with previously activated *Listeria* cultures, as outlined in section 2.1. The samples were then incubated at 37 °C for 48 hours.

Positive controls (culture medium inoculated with *Listeria* strains without NPAg) and blank controls (culture medium with NPAg at all studied concentrations, without *Listeria* strains) were also incubated. In each 96-well plate, 200 µL of microbial culture was added. After 48 hours of exposure to different NPAg concentrations, as described in section 2.2, microbial inhibition was assessed by spectrophotometry, using optical density (OD) as an indicator of cell growth. Measurements were performed at 595 nm using an iMark microplate reader (Bio-Rad).

2.4 Minimum Bactericidal Concentration Measurement

The determination of the Minimum Bactericidal Concentration (MBC) was performed using the macrodilution test with Demi Fraser Broth containing different NPAg concentrations (10–100%). Each tube, containing 9 mL of medium, was inoculated with 1 mL of bacterial suspension (10^6 CFU/mL) and incubated at 37 °C for 24 hours. After this period, visual assessment and deep plating on Tryptic Soy Agar were performed, followed by an additional 48-hour incubation to verify bacterial growth (+) or its absence (-).

The tubes were then re-incubated for another 24 hours, totaling 48 hours of exposure to NPAg, and the plating procedure was repeated to confirm the MBC. Positive (medium with bacterial inoculum) and blank (medium with NPAg without inoculum) controls were included to validate the results.

2.5 Experimental Design and Statistical Analysis

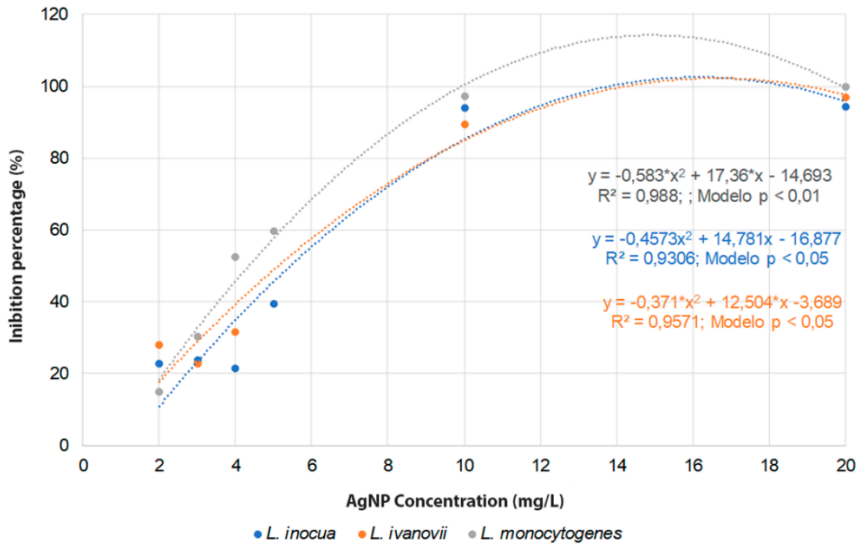
To evaluate the percentage reduction, a factorial experiment was conducted, considering NPAg concentrations of 2, 3, 4, 5, 10, and 20 mg/L and the species *L. ivanovii*, *L. innocua*, and *L. monocytogenes* as factors. The obtained data were subjected to analysis of variance (ANOVA), and the means were compared using the Scott-Knott test at a 5% significance level. The quantitative variable was evaluated using ANOVA for the model and regression indices. The analysis was conducted using the ExpDes.pt package (Ferreira; Cavalcanti; Nogueira, 2013) in the R software (R-Core Team, 2021).

3 Results and Discussion

3.1 Evaluation of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) test demonstrated the progressive efficiency of NPAg as the applied concentrations increased (Figure 1).

Figure 1 – Percentage (%) reduction of optical density after 48 hours of incubation



Source: research data.

The linear regression analysis for each bacterium in response to NPAg concentrations followed quadratic models, which were significant only for *L. innocua* and *L. monocytogenes*. These models demonstrated increasing inhibition up to concentrations between 11 and 15 mg/L, followed by a decrease at 20 mg/L. Inhibition at the highest concentration was greater for *L. monocytogenes* compared to the other two species, indicating that this strain was the most sensitive to NPAg action.

The results indicated that *L. innocua* and *L. ivanovii* strains exhibited greater resistance to NPAg when compared to *L. monocytogenes*.

Khan *et al*. (2021), using the microdilution test in plates, found results similar to those of the present study, with an MIC value of 15.62 mg/L for *L. monocytogenes* when exposed to colloidal silver.

Muthulakshmi *et al*. (2022) also observed that *L. monocytogenes* required high NPAg concentrations, above 50% (10 mg/L), for complete inhibition.

3.2 Minimum Bactericidal Concentration Measurement

Regardless of the minimum bactericidal concentration, there was no difference in response to nanoparticle exposure time. That is, for the same concentration, the observed effect was the same

after 24 or 48 hours of strain exposure (Table 1).

Table 1 - Minimum bactericidal concentration for *L. monocytogenes*, *L. innocua*, and *L. ivanovii* exposed for 24 and 48 hours to different NPAg concentrations.

NPAg Solution Concentration (mg/L)	Exposure Time (Hours)	*L. monocytogenes*	*L. innocua*	*L. ivanovii*
2	24	+	+	+
	48	+	+	+
3	24	+	+	+
	48	+	+	+
4	24	+	+	+
	48	+	+	+
5	24	-	+	+
	48	-	+	+
10	24	-	-	-
	48	-	-	-
20	24	-	-	-
	48	-	-	-

Legend: + No Inhibition, - Inhibition, result based on the average of three replicates.
Source: research data.

However, sensitivity varied according to the strain. For example, *L. monocytogenes* showed greater sensitivity to the NPAg solution, being inhibited from a concentration of 5 mg/L, while *L. innocua* and *L. ivanovii* were inhibited from 10 mg/L. It is important to highlight that the initial inoculum for all studied strains was standardized at approximately 10^8 CFU/mL.

Lianou *et al*. (2023), when analyzing the genome of *L. ivanovii*, identified the presence of the CRISPR protein (Clusters of Regularly Interspaced Short Palindromic Repeats), which is a defense mechanism found in the genes of some microorganisms, enabling greater resistance to stressful conditions, such as sanitization processes. This mechanism may be associated with the greater resistance of this strain compared to *L. monocytogenes* in the present study.

An inhibition concentration between 5 and 10 mg/L was also observed in a study conducted by Muthulakshmi *et al*. (2022), where silver nanoparticles also demonstrated the ability to inhibit protease production in *L. monocytogenes*, a key factor in virulence and propagation.

4 Conclusion

The colloidal silver solution exhibits antimicrobial activity against *Listeria* spp. strains.

The bacteriostatic and bactericidal activity against *Listeria* spp. strains increases proportionally with concentration.

The *L. innocua* strain demonstrates greater resistance.

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