UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Evaluation of Biofilm Eradication in Foodborne

Pathogens by Green Chemistry and Traditional Silver Nanoparticles.

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RESUMEN

Los biofilms, formados por patógenos transmitidos por alimentos como *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Bacillus cereus* y *Staphylococcus aureus*, presentan desafíos significativos debido a su resistencia a los tratamientos antimicrobianos convencionales. En este estudio se evaluó la eficacia de la erradicación de biofilms utilizando nanopartículas de plata (AgNPs) sintetizadas mediante métodos verdes y tradicionales. Las AgNPs verdes se produjeron utilizando extractos de plantas ecológicos, mientras que las AgNPs tradicionales emplearon reducción química con borohidruro de sodio ($NaBH_4$). Los resultados demostraron que las AgNPs tradicionales lograron tasas más altas de erradicación de biofilms a concentraciones más bajas. Por ejemplo, los biofilms de *S.* Typhimurium exhibieron una tasa de erradicación del 82.54% a 0.5 mM, mientras que las AgNPs verdes requirieron una concentración más alta de 5 mM para alcanzar una tasa de erradicación comparable del 81.95%. De manera similar, en *Escherichia coli*, las AgNPs tradicionales a 0.5 mM lograron una erradicación del 82.93%, mientras que las AgNPs verdes a 5 mM mostraron una tasa ligeramente inferior del 71.58%. En contraste, *Bacillus cereus* y *Staphylococcus aureus*, ambas bacterias Gram-positivas, demostraron mayor resistencia, requiriendo concentraciones más altas de AgNPs para una eliminación efectiva de biofilms. Estos resultados destacan la influencia de la estructura de la pared celular bacteriana en la eficacia del tratamiento. Este estudio subraya el potencial de las AgNPs, particularmente las variantes sintetizadas de manera ecológica, como alternativas sostenibles a los antimicrobianos convencionales. Si bien las AgNPs verdes requieren optimización para igualar la eficacia de los métodos tradicionales, su menor impacto ambiental y perfil de seguridad las convierten en candidatas prometedoras para el manejo de biofilms en la industria alimentaria y sectores relacionados.

Palabras claves: Erradicación de biofilm, Nanoparticulas de plata (AgNPs), Bacterias alimentarias

ABSTRACT

Biofilms, formed by foodborne pathogens such as *Escherichia coli, Salmonella enterica* serovar Typhimurium, *Bacillus cereus*, and *Staphylococcus aureus,* present significant challenges due to their resistance to conventional antimicrobial treatments. In this study, we evaluated the biofilm eradication efficiency of silver nanoparticles (AgNPs) synthesized through green and traditional methods. Green AgNPs were produced using eco-friendly plant extracts, while traditional AgNPs utilized chemical reduction with sodium borohydride ($NaBH_4$). The results demonstrated that traditional AgNPs achieved higher biofilm eradication rates at lower concentrations. For example, *S.* Typhimurium biofilms exhibited an 82.54% eradication rate at 0.5 mM, while green AgNPs required a higher concentration of 5 mM to achieve a comparable 81.95% eradication rate. Similarly, for *Escherichia coli,* traditional AgNPs at 0.5 mM achieved 82.93% eradication, whereas green AgNPs at 5 mM showed a slightly lower rate of 71.58%. In contrast, *Bacillus cereus* and *Staphylococcus aureus*, both Gram-positive bacteria, demonstrated greater resistance, requiring higher concentrations of AgNPs for effective biofilm removal. These results highlight the influence of bacterial cell wall structure on treatment efficacy. This study underscores the potential of AgNPs, particularly green-synthesized variants, as eco-friendly alternatives to conventional antimicrobials. While green AgNPs require optimization to match the efficacy of traditional methods, their reduced environmental impact and safety profile make them promising candidates for biofilm management in food processing and related industries.

Keywords: Biofilms eradication, Silver nanoparticles (AgNPs), Foodborne bacteria

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INTRODUCTION

Biofilms are structured communities of microorganisms embedded within a self-produced extracellular polymeric substance (EPS) matrix, adhering to surfaces and resisting environmental stresses. (Carrascosa et al., 2021; Jara-Medina et al., 2024). This protective matrix enables microbes to thrive under adverse conditions, such as UV exposure, dehydration, lack of nutrients, and antimicrobial agents among others, making biofilms a significant concern in environmental, industrial, and medical contexts (Pang et al., 2023; Zhou et al., 2022). Biofilms can be formed on biotic and abiotic surfaces, including food processing equipment, medical devices, and living tissues, posing severe public health risks due to their enhanced antimicrobial resistance (Galié et al., 2018; Cangui-Panchi et al., 2023; Cangui-Panchi et al., 2022).

Biofilms can harbor various pathogenic bacteria, including *Escherichia coli, Salmonella enterica* serovar Typhimurium, *Bacillus cereus,* and *Staphylococcus aureus*, which present unique challenges due to their resistance profiles and survival strategies. *Escherichia coli* is a typical and commensal Gram-negative bacterium commonly associated with gut microbiota. However, certain pathogenic strains, known as *E. coli* pathotypes, can cause foodborne illnesses (Cangui-Panchi et al., 2023). Its ability to form biofilms on living and non-living surfaces enhances its resistance to environmental stresses and disinfectants, making cross-contamination in food processing environments a persistent issue. Pathotypes, such as enterohemorrhagic *Escherichia coli*, can cause severe gastrointestinal diseases, including hemolytic uremic syndrome (Zhou et al., 2022). Biofilm formation facilitates *E. coli*'s survival, even under rigorous sterilization protocols (Galié et al., 2018). Another genus containing pathogenic serovars is *Salmonella* causing global foodborne outbreaks. Most Gram-negative pathogens are able to form resilient biofilms in diverse environments, including food processing plants (Kundu et al., 2024). *Salmonella enterica* serovar Typhimurium is one of the most prevalent serovars implicated in global foodborne illnesses. This bacterium is highly adept at forming biofilms, particularly on food processing surfaces such as stainless steel,

polyethylene, and other industrial materials (Pang et al., 2023). Its biofilm matrix is primarily composed of curli fimbriae and cellulose that not only facilitates adhesion to surfaces but also enhances resistance to environmental stressors, including disinfectants and desiccation (Carrascosa et al., 2021). The biofilm's protective structure enables *S.* Typhimurium to persist under harsh conditions, leading to contamination of raw and processed foods, such as poultry, eggs, and dairy products. Moreover, the pathogen's ability to withstand sublethal concentrations of antimicrobials often results in increased resistance and cross-protection against other disinfectants such as oxidizing agents, denaturing agents, surfactants, and enzyme-based compounds (Pang et al., 2023). The persistence and adaptability of *S.* Typhimurium in biofilms pose significant challenges in ensuring food safety and public health, emphasizing the need for innovative control strategies.

Bacillus cereus, a Gram-positive bacterium, is also able to establish biofilms in submerged or surface environments. These biofilms can secrete toxins such as hemolysins HlyI and HlyII, and enzymes like proteases and phospholipases, which cause spoilage and foodborne illnesses (Majed et al., 2016). Moreover, its ability to form highly adhesive spores contributes to its biofilm resilience, making it resistant to thermal processes (Galié et al., 2018). Another Gram-positive opportunistic pathogen is *Staphylococcus aureus* forming robust biofilms on biotic and abiotic surfaces, like mucosal membranes, skin, and industrial materials like stainless steel. *S. aureus* biofilms are particularly resistant to antimicrobial agents due to their high EPS content, which includes polysaccharide intercellular adhesin and several proteins like fibrinogen-binding protein (Idrees et al., 2021; Galié et al., 2018). These biofilms also contribute to food contamination and complicated clinical treatments.

Therefore, several treatments are applied to eradicate these pathogenic biofilms during food processing. One of the suggested alternative treatments for biofilm eradication is the application of nanoparticles, particularly silver nanoparticles (AgNPs), which have emerged as promising agents for biofilm control (Liu et al., 2023). Traditional synthesis of AgNPs often employs chemical methods, such as the use of sodium borohydride as a reducing agent. However, these methods are energy-intensive, hazardous, and produce toxic by-products,

limiting their environmental sustainability and safety for food industry applications (Ratan et al., 2021). In nanotechnology, green chemistry has partially replaced traditional synthesis methods, which are energy-intensive, hazardous, and generate toxic by-products. Green synthesis of nanomaterials, using clean and eco-friendly methods, has gained popularity due to its simplicity and reliance on biological systems. This approach has facilitated the application of nanoparticles in fields such as environmental remediation, photocatalysis, sensors, solar cells, and energy storage (Souza et al., 2021). Also, the biocompatibility and antimicrobial properties of these nanoparticles have enabled their use in biomedical applications, including diagnostics, wound healing, immunotherapy, regenerative medicine, and targeted drug delivery. Nonetheless, challenges remain in selecting green raw materials, optimizing synthesis conditions, ensuring quality and stability, and assessing long-term properties for future use (Souza et al., 2021). Green AgNPs could enhance biofilm inhibition and eradication, particularly against Gram-negative bacteria. Their small size and high surface area enable efficient penetration and interaction with microbial cells, disrupting biofilm integrity (Shakir, 2016). So, the present study aims to compare the antibiofilm activity between AgNPs from traditional and green methodologies against well-known foodborne pathogens.

METHODS

Bacteria isolates and growth conditions

Silver nanoparticles on green chemistry were studied for the ability to eradicate biofilm formation against foodborne bacteria, specifically Gram-positive bacteria such as *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923, and Gram-negative bacteria as *Escherichia coli* ATCC 25922 and *Salmonella enterica* serovar Typhimurium ATCC 14028 from the bacterial collection of Institute of Microbiology of the Universidad San Francisco de Quito (IM-USFQ). All bacterial species were stored at -80 °C and grown on Mueller-Hinton agar (MHA) medium at 37 °C for 24 hours before each assay.

Biofilm eradication assays

A culture of the bacterial species was grown overnight, followed by the preparation of an inoculum with a concentration of $1x10^7$ colony-forming units (CFU)/mL using tubes with 7 mL of saline solution, based on an initial 0.5 McFarland turbidity standard (equivalent to $1x10^8$ CFU/mL), and then diluted by a factor of 10. The inoculum was then centrifuged at 400 rpm for 10 minutes, and the pellet was re-suspended in the same volume of sterile Mueller-Hinton broth (MHB). Afterward, 200 µL of inoculum plus media were added to each well, except the negative control (containing only the media). The plate was then incubated at 37°C for 24 hours under aerobic conditions to allow biofilm formation. Following incubation, the media was carefully removed and replaced with 200 µL of sterile media. The biofilms were then treated with fresh media containing 1× and 2× of the previously determined minimum inhibitory concentrations (MIC) from each antimicrobial treatment including: Green (AgNPs), Traditional (AgNPs), Silver ions (Ag+), and $NaBH_4$ (Cabascango, 2023). The plate was incubated once more at 37°C for 24 hours under aerobic conditions, and the wells were gently washed with phosphate-buffered saline (PBS; at pH 7.4) through a 45-degree angle, avoiding contact with the bottom of the wells. Depending on the pathogen and protocol, one or two additional washing steps with PBS (pH 7.4) were performed.

Biomass evaluation

To evaluate biomass eradication by the different nanoparticle treatments, the wells in the 96-well plates were washed once with 200 μL of PBS. Optical density (OD) values were measured at 570 nm using a microplate spectrophotometer (ELISA Elx808, Biotek, Winooski, USA), as previously outlined by Atiencia et al. (2022). This method showed no significant difference between crystal violet (CV) staining and PBS suspension techniques for measuring biomass formation, based on a modified version of Gulati et al. (2018)'s method.

Statistical analysis

All experimental data was statistically analyzed on RStudio software through non-parametric tests such as the Wilcoxon test. It was performed to compare differences between positive control and both treatments/buffer/Ag ions during the biofilm eradication assays. The applied R software was the newly released version on September 23, 2024, from the official web page (https://posit.co/download/rstudio-desktop/) using the following libraries packages: "rstatix", "readxl", "dplyr", "data.table", and "knitr".

RESULTS

Evaluation of biofilm eradication on foodborne pathogens

The analysis of biofilm eradication in foodborne bacteria using silver nanoparticles synthesized through green chemistry (green AgNPs) versus traditional methods revealed significant results across different bacterial strains. More exactly, concerning *Bacillus cereus* ATCC 11778, the green AgNPs at 5.0 mM (2× MIC) demonstrated 72.62% biofilm eradication (p-value = 3.38e-05), while the traditional AgNPs treatment at 1.0 mM (2× MIC) eradicated 70.02% of biofilm (p-value = 3.38e-05). Ag^+ ions at 1.0 mM showed 69.51% eradication (p-value = 3.42e-05), while the lowest efficacy was again observed with $NaBH_4$ (buffer) at 1.0 mM, which achieved only 16.60% eradication (p-value = 2.56e-03) (Table 1).

Meanwhile, when evaluating *Staphylococcus aureus* ATCC 25923, The green AgNPs at 2.5 mM (2× MIC) demonstrated 53.10% of biofilm eradication (p-value = 3.6e-05) but showed a lower percentage of 41.61% at 1.0 mM (1× MIC) with a (p-value = 3.59E-02). Traditional AgNPs at 1.0 mM (2× MIC) and 0.25 mM (0.5× MIC) yielded superior eradication results of 78.13% (p-value = 3.57e-05) and (p-value=3.57e-05). Ag^+ ions at 1.0 mM showed an eradication rate of 78.13% (p-value = 3.59e-05), being similar to traditional AgNPs at 1.0 mM. As expected, $NaBH_4$ (buffer) exhibited the lowest eradication percentage, with only 18.16% eradicated (p-value = 2.43e-04), as shown in Table 1.

Concerning *Escherichia coli* ATCC 25922, the green AgNPs at 5.0 mM (2× MIC) showed a remarkable eradication rate of 71.58%, while the green AgNPs at 2.5 mM concentration (1× MIC) eradicated 55.26%. Both concentrations evidenced highly significant eradication values when compared to controls (Table 2), more precisely, p-values of 3.55e-05 and 3.59e-05, respectively. On the other hand, the traditional AgNPs at 0.5 mM exhibited an eradication efficacy of 82.93% with a p-value of 3.57e-05, showing comparable effectiveness to the green AgNPs. In comparison, Ag^+ ions at 0.5 mM eradicated 59.49% (p-value = 3.60e-05), demonstrating moderate efficacy. $NaBH_4$ buffer showed the lowest eradication percentage at 40.42% (p-value = 3.59e-05). As expected, the negative controls showed no biofilm

eradication, thus validating the experimental evaluation of the present study.

Regarding *Salmonella Typhimurium* ATCC 14028, the green AgNPs treatment at 5.0 mM resulted in an eradication rate of 81.95% (p-value = 3.40e-05), while the traditional AgNPs at 0.5 mM (2× MIC) achieved a slightly higher efficacy with 82.54% biofilm eradication (p-value = 3.35e-05). Ag^+ ions at 0.5 mM showed an eradication rate of 71.73% (p-value = 3.26e-05), slightly lower than the green and traditional AgNPs. In contrast, $NaBH_4$ buffer exhibited again the lowest eradication efficacy at 21.92% (p-value = 3.41e-05). Lower concentrations of the traditional AgNPs showed decreased efficacy, as expected (see Table 2).

Overall, the present findings underscore the effectiveness of both green and traditional AgNPs, when compared to Ag^+ ions, in biofilm eradication across various bacterial pathogens with statistically significant results. In contrast, $NaBH_4$ buffer consistently showed the lowest eradication efficacy across all strains, reinforcing its limited antimicrobial activity. These results highlight the potential of green and traditional AgNPs as viable antimicrobial agents in foodborne pathogen management.

DISCUSSION

Silver nanoparticles (AgNPs) have exhibited substantial potential in reducing mature biofilms formed by both Gram-positive and Gram-negative foodborne bacteria. The results shown in Figures 1 and 2 illustrate the differences in biofilm eradication efficiency among the four bacterial strains, with the variance largely attributed to the structural differences between Gram-positive and Gram-negative bacteria. The thick peptidoglycan layer characteristic of Gram-positive bacteria could pose a significant barrier, thus reducing the efficacy of eradication when AgNPs are used alone (Vaiwala et al., 2022). However, studies have shown that combining AgNPs with hydrogels, plant extracts, bee/probiotic-derived products, and/or antibiotics can enhance their antimicrobial activity (Miño et al., 2024; Machado et al., 2023), achieving up to 85% biofilm reduction in *Staphylococcus aureus* at 2× MIC (2.5 mM) (Bouryabaf et al., 2017). This study underscores the effectiveness of AgNPs, particularly against biofilm-forming bacteria that are inherently resistant to conventional antibiotics. A critical factor in their success is the ability of AgNPs to penetrate the extracellular polymeric substances (EPS) of biofilms, which serve as a protective matrix for bacterial cells and significantly limit the efficacy of traditional antimicrobial agents (Idrees et al., 2021). Collectively, these studies highlight the promising role of AgNPs in overcoming biofilm-associated infections and emphasize the need for continued research into their mechanisms of action and optimized application strategies.

When it comes to eradicating *Bacillus cereus,* AgNPs already have shown remarkable efficacy, achieving a 5-log reduction in planktonic bacterial populations such as *Staphylococcus aureus, Listeria innocua, Salmonella enterica* serovar Choleraesuis, *Pseudomonas aeruginosa, Escherichia coli,* including *B. cereus (*Araújo et al., 2012). This is particularly notable given the resilience of *B. cereus*, a Gram-positive bacterium with a thick peptidoglycan layer and the ability to form spores that are resistant to environmental stresses. Silver nanoparticles synthesized chemical composition such as $NaBH_4$ or dodecyltrimethylammonium bromide (Dotab), which have been particularly effective in

overcoming these structural defenses, making them a strong candidate for use in industrial and clinical sanitation protocols (Araújo et al., 2012). These findings underline the versatility of AgNPs in addressing Gram-positive bacteria, which are often more challenging to eliminate due to their structural complexities.

On the other hand, a comparative analysis between AgNPs and sodium hypochlorite (NaOCl) further demonstrates the superior efficacy of AgNPs. While NaOCl requires concentrations as high as 29.6 mM to achieve antimicrobial effects, AgNPs achieve similar results at only 5 mM, offering a more efficient and environmentally sustainable solution (Ismail et al., 2019). Furthermore, AgNPs leave fewer toxic residues, making them more suitable for applications where safety is paramount. This efficiency, combined with their broader spectrum of action, positions AgNPs as a promising alternative to traditional disinfection methods in various industries.

For Gram-negative bacteria, such as *Escherichia coli*, eradication rates exceeding 80% have been achieved using silver nanoparticles AgNPs. A study demonstrated that green-synthesized AgNPs derived from *Azadirachta indica* extracts consistently inhibited and eradicated *E. coli* biofilms, evidencing eradication rates of 84–96% with higher concentrations of 3.32 mM (2× MIC) (Zena & Alaa, 2023). In comparison, the results of the study show that green AgNPs at higher concentrations of 5 mM achieved a biofilm eradication rate of 71%, indicating that higher concentrations may not always correlate with increased efficacy. However traditional AgNPs demonstrated a rate of 82% biofilm eradication at much lower concentrations of 0.25 and 0.50 mM, showcasing their ability to perform effectively even at reduced dosages (More et al., 2023). In contrast to *E. coli*, biofilms formed by *Salmonella enterica* serovar Typhimurium are notably more resilient, requiring higher nanoparticle concentrations or prolonged exposure times for effective eradication. This resilience is attributed to the presence of cellulose and curli fimbriae, which enhance the mechanical and chemical resistance of *S.* Typhimurium biofilms against antimicrobial agents (Ramachandran et al., 2016). For instance, treatments with silver nanoparticles achieved a 60% biofilm reduction at a higher concentration (2× MIC) of 125 µg/mL (~1.16 mM) (Bouryabaf et al.,

2017). These findings suggest that while dosage adjustments may be necessary, AgNPs remain an effective solution for controlling Gram-negative bacterial biofilms. Additionally, nanoparticle size plays a critical role, with smaller particles demonstrating superior biofilm penetration and enhanced antimicrobial activity due to their higher surface-area-to-volume ratios (Kundu et al., 2024).

Optimizing nanoparticle size is crucial for maximizing biofilm disruption, as smaller AgNPs have been shown to effectively inhibit the synthesis of key components in biofilm matrices, thereby limiting bacterial colonization and persistence. Additionally, green-synthesized AgNPs, produced using environmentally friendly reducing agents from plant extracts, offer a significant advantage over traditional AgNPs synthesized with toxic compounds like sodium borohydride ($NaBH_4$) (Younus et al., 2024). Green synthesis not only reduces environmental and health risks but also eliminates the need for hazardous chemicals, making the nanoparticles safer for industrial and clinical applications (More et al., 2023). Furthermore, AgNPs, including green variants, exhibit superior efficacy compared to traditional disinfection methods, achieving high biofilm inhibition rates at lower concentrations. This efficiency reduces operational costs, minimizes environmental impact, and effectively tackles the challenges posed by mixed microbial biofilms prevalent in food processing and healthcare settings (Cangui-Panchi et al., 2022; Galie et al., 2018). These combined benefits: enhanced efficacy, lower toxicity, and environmental sustainability. So, the green AgNPs represent a compelling and innovative solution for biofilm-related challenges in diverse applications.

CONCLUSIONS

Both traditional and green-synthesized silver nanoparticles (AgNPs) have demonstrated significant efficacy in eradicating biofilms, confirming their potential as powerful antimicrobial agents in the present study. While traditional AgNPs require lower concentrations to achieve biofilm eradication, green AgNPs offer a more sustainable and eco-friendly alternative. The need for higher concentrations of green AgNPs indicates the need for further research in optimizing the green synthesis of AgNPs for food safety and effectively achieving higher biofilm eradication on food-contact surfaces or products. Their environmental benefits make them an attractive option in comparison to other treatments. Future research should focus on assessing biofilm formation over extended periods (48 or 72 hours) and testing under varying conditions, including combined treatments and against other microorganisms such as fungi. Additionally, evaluating the cytotoxic effects of both traditional and green AgNPs in the food industry is crucial to ensure safety while advancing the adoption of sustainable antimicrobial strategies.

TABLES

Table 1. Evaluation of biofilm eradication in Gram-positive foodborne pathogenic bacteria.

Microorganism	Component	Type of concentration	Eradication (%)	Biomass (%)	P- value	Level of significance
Bacillus cereus ATCC 11778	Green AgNPs	5.0 mM	72.62	27.38	3.38e-05	***
		2.5 mM	51.83	48.17	3.41e-05	***
	Ag^+ ions	1.0 mM	69.51	30.49	3.42e-05	***
		0.5 mM	40.43	59.57	3.41e-05	***
	Traditional AgNPs	1.0 mM	70.02	29.99	3.38e-05	***
		0.5 mM	56.30	43.70	3.37e-05	***
	$NaBH_4$ buffer	1.0 mM	16.60	83.40	2.56e-03	**
		0.5 mM	18.52	81.48	1.477e-05	***
	Control (+)	-	0.00	100.00	-	
	Control (-)	-	100.00	0.00	-	
Staphylococcus aureus ATCC 25923	Green AgNPs	2.5 mM	53.10	46.90	3.6e-05	***
		1.0 mM	41.61	58.39	3.59e-02	*
	Ag^+ ions	1.0 mM	78.13	21.87	3.59e-05	***
		0.5 mM	65.54	34.47	3.56e-05	***
	Traditional AgNPs	1.0 mM	78.13	21.87	3.57e-05	***
		0.5 mM	62.16	37.84	3.57e-02	*
	$NaBH_4$ buffer	1.0 mM	18.16	89.77	2.43e-04	***
		0.5 mM	10.23	81.84	1.01e-02	*
	Control (+)	-	0.00	100.00	-	
	Control (-)	-	100.00	0.00	-	

Legend: Eradication percentages on Gram-positive on each treatment: Green AgNPs, Ag^+ ions, Traditional AgNPs, and buffer. Also, the statistical analysis was realized illustrating p-values as *** when $\alpha \leq 0.001$, ** when $\alpha \leq 0.01$, and * when $\alpha \leq 0.05$.

Table 2. Evaluation of biofilm eradication in Gram-negative foodborne pathogenic bacteria.

Microorganism	Component	Type of concentration	Eradication (%)	Biomass (%)	P- value	Level of significance
Escherichia coli ATCC 25922	Green AgNPs	5.0 mM	71.58	28.42	3.55e-05	***
		2.5 mM	55.26	44.74	3.59e-05	***
	Ag^+ ions	0.5 mM	59.49	40.51	3.6e-05	***
		0.25 mM	54.33	45.67	3.59e-05	***
	Traditional AgNPs	0.5 mM	82.93	31.53	3.57e-05	***
		0.25 mM	68.48	17.08	3.57e-05	***
	$NaBH_4$ buffer	1.0 mM	40.42	59.58	3.59e-05	***
		0.5 mM	23.11	76.89	3.56e-05	***
	Control (+)	-	0.00	100.00	-	
	Control (-)	-	100.00	0.00	-	
Salmonella Typhimurium ATCC 14028	Green AgNPs	5.0 mM	81.95	18.05	3.40e-05	***
		2.5 mM	70.60	29.40	3.42e-05	***
	Ag^+ ions	0.5 mM	71.73	28.27	3.26e-05	***
		0.25 mM	66.90	33.10	3.42e-05	***
	Traditional AgNPs	0.5 mM	82.54	17.46	3.35e-05	***
		0.25 mM	52.48	47.52	3.41e-05	***
	$NaBH_4$ buffer	1.0 mM	21.92	78.08	3.41e-05	***
		0.5 mM	19.20	80.80	2.54e-03	**
	Control (+)	-	0.00	100.00	-	
	Control (-)	-	100.00	0.00	-	

Legend: Eradication percentages on Gram-positive on each treatment: Green AgNPs, Ag^+ ions, Traditional AgNPs, and buffer. Also, the statistical analysis was realized illustrating p-values as *** when $\alpha \leq 0.001$, ** when $\alpha \leq 0.01$, and * when $\alpha \leq 0.05$.

FIGURES

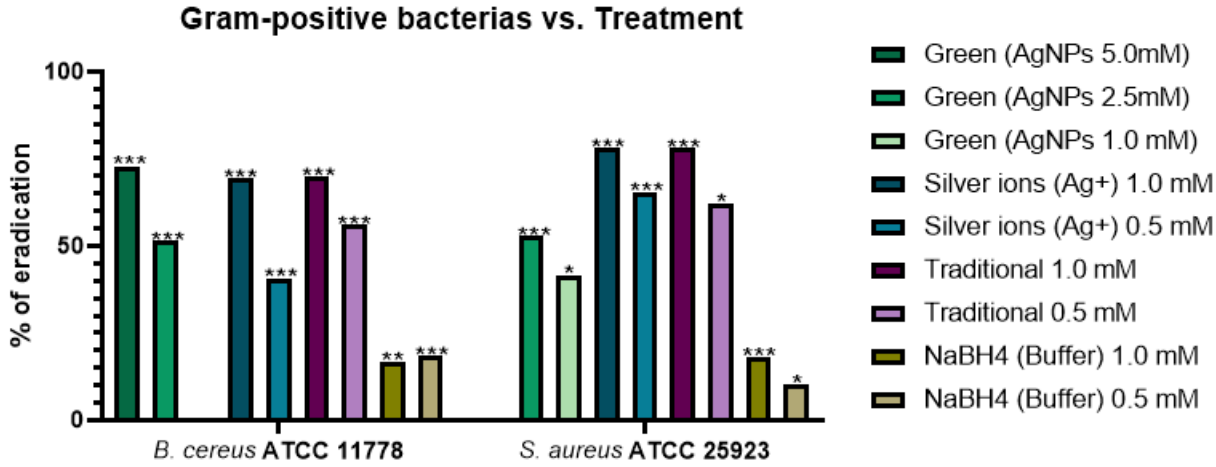

Figure 1. Biofilm eradication efficacy of various treatments against Gram-positive bacteria. The bar graph illustrates the percentage of biofilm eradication in *Bacillus cereus* ATCC-11778 and *Staphylococcus aureus* ATCC-25923 following treatment with different antimicrobial agents. Treatments include green silver nanoparticles (AgNPs) at concentrations of 5.0 mM, 2.5 mM, and 1.0 mM; silver ions (Ag^{+}) at 1.0 mM and 0.5 mM; traditional silver treatments at 1.0 mM and 0.5 mM; and $NaBH_4$ buffer at 1.0 mM and 0.5 mM. The positive control represents complete biofilm presence without treatment intervention. Statistical significance relative to positive control is indicated by asterisks (***p-value < 0.001, **p-value < 0.01, *p-value < 0.05). This data highlights the varying efficacy of each treatment in biofilm reduction for these Gram-positive pathogens.

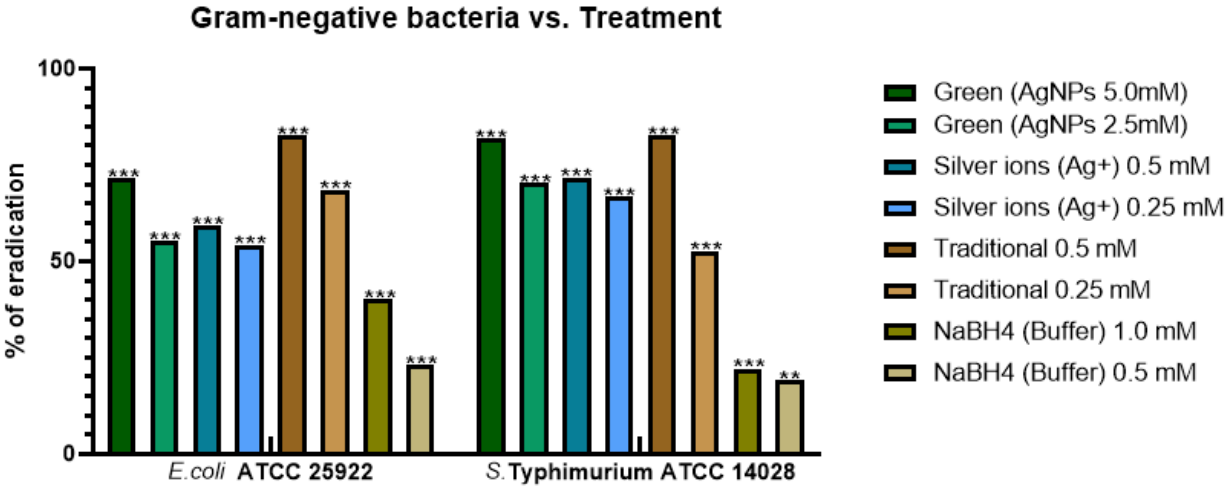

Figure 2. Biofilm eradication efficacy of various treatments against Gram-negative bacteria. The bar graph displays the percentage of biofilm eradication in *Escherichia coli* ATCC-25922 and *Salmonella enterica* serovar Typhimurium ATCC-14028 under different antimicrobial treatments. Treatments include green silver nanoparticles (AgNPs) at concentrations of 5.0 mM and 2.5 mM, silver ions (Ag^{+}) at 0.5 mM and 0.25 mM, traditional treatment at 0.5 mM and 0.25 mM, and $NaBH_4$ buffer at 1.0 mM and 0.5 mM. The positive control represents baseline biofilm presence without treatment. Statistical significance relative to positive control is denoted by asterisks (***p-value < 0.001, **p-value < 0.01), indicating treatments that produced significantly different eradication percentages. The results highlight the comparative effectiveness of each treatment modality in reducing biofilm formation in these Gram-negative pathogens.

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