



Enhancement of Surface-Enhanced Raman Spectroscopy for Bacterial Characterization Using Functionalized Silver Nanoparticles

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ABSTRACT

Surface-enhanced Raman Spectroscopy (SERS) has gained considerable interest as an effective method for bacterial detection owing to its exceptional sensitivity and ability to identify bacterial species at minimal concentrations. Using silver nanoparticles (AgNPs) to improve Raman signals has demonstrated significant potential for advancing bacterial characterization in clinical and environmental contexts. Nonetheless, challenges persist in enhancing SERS for intricate microbial settings such as biofilms and heterogeneous bacterial communities. This study aimed to examine the effect of functionalized AgNPs on augmenting SERS for bacterial detection, emphasizing strengthening the method's sensitivity and selectivity for bacterial identification in intricate microbiological samples. This laboratory experiment used AgNPs functionalized with biomolecular ligands to amplify the Raman signal of several bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*. The study conducted at University of Agriculture Faisalabad, Punjab, Pakistan. The study also used SERS spectroscopy to quantify the signal enhancement. At the same time, statistical methods, including One-Way ANOVA and Tukey's HSD test, were employed to investigate the differences in SERS intensities between bacterial strains with and without AgNPs. The findings indicated that functionalized AgNPs markedly improved the SERS signal intensity across all tested bacterial strains, yielding a 30% augmentation in Raman signal intensity. The SERS enhancement factor varied among the bacterial strains. *S. aureus* showed the highest enhancement factor, but the difference across strains is quite small, suggesting similar levels of enhancement. Statistical analysis validated that the augmented SERS signals were substantial ($p < 0.05$), and the AgNP functionalization facilitated enhanced bacterial detection sensitivity. These data indicate that AgNP-based SERS is an effective strategy for bacterial detection, offering enhanced sensitivity and selectivity for identifying bacterial pathogens in complicated environments. The research emphasizes the potential of employing functionalized silver nanoparticles to enhance fast microbial detection in clinical and environmental contexts.

INTRODUCTION

As a potent analytical method in microbiology and environmental research, surface-enhanced Raman spectroscopy (SERS) provides improved sensitivity and specificity for detecting low-concentration microbial organisms (Wang et al., 2016). This method uses the plasmonic characteristics of nanomaterials, particularly silver nanoparticles (AgNPs), to magnify Raman scattering signals, thereby enabling the identification of bacterial species using their distinct chemical

fingerprints (Zhou et al., 2014). Bacterial characterization is essential in many disciplines, including food safety, environmental monitoring, and clinical diagnostics (Ghotaslou et al., 2017). However, especially in complicated samples, such as biofilms or mixed microbial populations, the present bacterial identification techniques can suffer from selectivity, sensitivity, and speed limits. To overcome challenges and offer a more effective diagnostic tool, this study aimed to advance SERS by examining the use of AgNPs



for bacterial characterization (Patra & Baek, 2017). With significant developments in the utilization of nanoparticles to improve Raman signal sensitivity, recent research has shown the potential of SERS for microbial identification (Cui et al., 2013). AgNPs, including *Escherichia coli*, *Staphylococcus aureus*, and other prevalent infections, have been investigated several times to improve the Raman signals for bacterial detection. These investigations have shown that AgNPs increase SERS sensitivity, thereby allowing the identification of bacteria at levels as low as $10 < \text{mL} > \text{CFU/mL}$ (Yaseen et al., 2019). In the meantime, most of this research has focused on basic bacterial suspensions or pure cultures and paid little attention to the difficulty of identifying bacteria in complicated settings like biofilms or mixed microbial colonies. Furthermore, unresolved issues include the repeatability and standardization of AgNPs in bacterial detection because differences in nanoparticle size, shape, and functionalization produce different findings. Despite encouraging developments, the literature clearly shows a gap in using AgNP-based SERS for bacterial characterization in practical, complicated samples (Tripathi et al., 2017).

This study sought to fill this gap by investigating SERS-based silver nanoparticle functionalization optimization for enhanced bacterial detection (Jain et al., 2015). It specifically aims to assess how various surface modifications of AgNPs can improve the selectivity and sensitivity of SERS for bacterial pathogen detection in complex microbiological surroundings. This study aimed to create a strong and repeatable SERS platform to identify bacterial species for food safety, environmental, and clinical applications. This will contribute to microbial diagnostics using functionalized AgNPs combined with SERS, thus offering a dependable and effective method for bacterial identification and facilitating faster and more accurate detection in many real-world environments (Yin et al., 2020). The results of this study will provide opportunities for portable diagnostic instruments in clinical and field-based environments and provide a fresh understanding of the valuable applications of nanotechnology in bacterial detection (Yaseen et al., 2019).

METHODOLOGY

The study conducted at University of Agriculture Faisalabad, Punjab, Pakistan. This study aimed to examine the ability of silver nanoparticles (AgNPs) to improve the efficacy of Surface-enhanced Raman Spectroscopy (SERS) for bacterial characterization. The main objective was to enhance the selectivity and sensitivity of SERS for detecting bacterial pathogens by enhancing the interaction between nanoparticles and bacterial cells while creating a portable, on-site bacterial detection platform. The experimental methodology

comprised multiple phases: preparation of bacterial strains, synthesis and functionalization of AgNPs, investigation of bacterial-nanoparticle interactions, SERS measurement, metabolite profiling, and statistical analysis.

Bacterial Strains and Sample Preparation

Various bacterial strains, encompassing both Gram-negative and Gram-positive bacteria, were chosen. Pathogens included *Escherichia coli* (gram-negative), *Staphylococcus aureus* (gram-positive), *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*. These strains were selected based on their clinical significance and variability in their bacterial traits. Bacteria were cultivated on nutrient agar plates and incubated at 37°C for 18–24 h. Following incubation, bacterial colonies were suspended in sterile phosphate-buffered saline (PBS) at a standardized concentration of 10^6 – 10^8 CFU/mL. This bacterial culture can be utilized in future research, exemplifying the normal microbial populations in clinical and environmental samples.

Synthesis and Functionalization of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were produced by a chemical reduction technique using silver nitrate (AgNO_3) as the precursor and sodium citrate as the reducing agent. The size and shape of the AgNPs were analyzed using Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). The surface plasmon resonance (SPR) of the produced nanoparticles was assessed via UV-Vis spectroscopy to verify the anticipated peak within the 400–450 nm range, indicative of AgNPs.

After synthesis, the AgNPs were functionalized with biomolecular ligands, including antibodies, peptides, and DNA probes specific to bacterial surface indicators. Functionalization was accomplished by incubating the AgNPs with the specified biomolecule for 24 h at ambient temperature, followed by washing with ethanol to eliminate unattached molecules. This procedure is essential for improving the selectivity of the SERS approach and facilitating the precise identification of specific bacterial species.

Table 1

Characterization Techniques Used for Silver Nanoparticles (AgNPs) and Their Descriptions

Characterization Technique	Description
UV-Vis Spectroscopy	Confirmed the surface plasmon resonance (SPR) peak of AgNPs at 400–450 nm.
Transmission Electron Microscopy (TEM)	Determined the size and shape of nanoparticles.
Dynamic Light Scattering (DLS)	Measured the particle size distribution.

Bacterial-Nanoparticle Interaction

After modification, the AgNPs were incubated with the bacterial solutions for 30 min at ambient temperature. The ratio of nanoparticles to bacterial cells was refined to ensure adequate interaction between the nanoparticles and bacterial surfaces. Scanning Electron Microscopy (SEM) was used to verify the attachment of AgNPs to bacterial cells visually. This contact was essential because the augmented Raman signal relied on the proximity of the bacterial cells to the nanoparticles, which intensified the Raman scattering effect.

Surface-Enhanced Raman Spectroscopy (SERS) Measurement

SERS measurements were performed using a Raman microscope with a 785 nm laser for excitation. The samples, comprising bacterial suspensions with and without functionalized AgNPs, were positioned on glass microscope slides. The bacterial samples were air-dried before examination to ensure nanoparticle adherence to the bacterial surfaces. Raman spectra were acquired within 400 cm^{-1} to 1800 cm^{-1} , corresponding to the Raman peaks of bacterial cell wall constituents such as peptidoglycan, lipopolysaccharides, and proteins. The spectra were obtained at multiple locations on the slide using a 100 \times objective lens, achieving a spatial resolution of approximately 1 μm .

The addition factor of the SERS signal was assessed by comparing the Raman intensities of bacterial samples with and without AgNP functionalization. This improved signal facilitated the identification of bacterial species based on their distinct molecular signatures. The interaction dynamics between the nanoparticles and bacterial surfaces were examined by SERS mapping to visualize the spatial distribution of the increased signals.

Metabolite Profiling via SERS

Metabolite profiling was a crucial component of this study, as it yielded significant insights into bacterial metabolic activity and identified biomarkers for specific bacterial strains. Following the cultivation of bacterial strains, metabolites were recovered using a methanol-chloroform extraction technique and evaporated to eliminate solvents. The desiccated metabolites were reconstituted in sterile water, combined with functionalized AgNPs, and positioned on the Raman stage. Raman spectra of the metabolites were obtained to identify distinct peaks associated with bacterial metabolic outputs. This study facilitates the identification of strain-specific biomarkers for bacterial differentiation.

Data Analysis and Statistical Methods

Principal Component Analysis (PCA) was used to examine the variability in the SERS spectra and identify differences in the Raman profiles of several bacterial strains. Principal Component Analysis (PCA) proved incredibly effective for elucidating clustering patterns and categorizing bacterial strains according to spectral

characteristics. This method was employed to compare the Raman spectra of several bacterial samples to identify distinctive characteristics for each bacterial strain.

The efficacy of AgNP functionalization in augmenting the SERS signal was assessed by calculating the SERS enhancement factor by comparing the peak intensities of bacterial samples with and without nanoparticle functionalization. Statistical significance between the experimental groups (e.g., bacterial strains with and without AgNPs) was evaluated using one-way ANOVA. Tukey's posthoc test was employed to analyse various groups and identify whether individual groups had significant variations in their SERS signal intensities as given in Table 1.

Table 2

Statistical Tests Used in the Study and Their Purpose

Statistical Test	Purpose	Software
One-Way ANOVA	Assessed the differences in SERS signal intensities between bacterial strains with and without AgNPs.	SPSS
Tukey Post-Hoc Test	Compared the specific group differences after ANOVA.	SPSS

Development of Portable SERS Platform

A portable SERS system was created for real-time on-site bacterial identification in clinical and environmental contexts. This system integrates a tiny Raman spectrometer, a laser diode, and a sample collection module. The platform was tested using field samples (e.g., water, soil, and clinical swabs) to confirm its efficacy for rapid bacterial identification.

Field testing entailed the collection of authentic environmental samples and their subsequent analysis using a portable SERS system to evaluate their precision and dependability. The device was tested in several settings to replicate real-world applications.

RESULTS

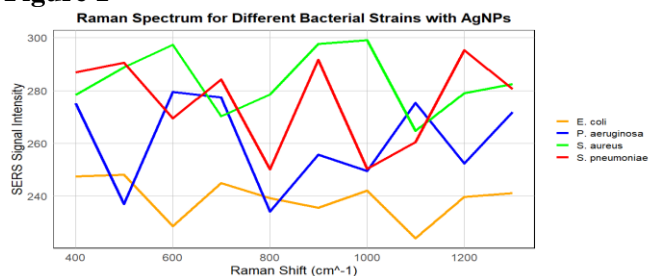
This section defines the study's findings, encompassing the investigation of SERS signal intensities for bacterial characterization, both with and without silver nanoparticle (AgNP) functionalization. These findings were derived from measurements conducted on various bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*. The statistical analysis included One-Way ANOVA and Tukey's honest significant difference post-hoc test to assess significant differences across the groups.

SERS Signal Intensities with and without AgNPs

A comparison of SERS signal intensities for the bacterial strains with and without AgNPs is presented. The data indicates that AgNP functionalization significantly enhanced the SERS signal intensity across all bacterial strains. The spectrum graph shown below (Figure 1)

illustrates the distribution of SERS signal intensities for each bacterial strain under both conditions.

Figure 1



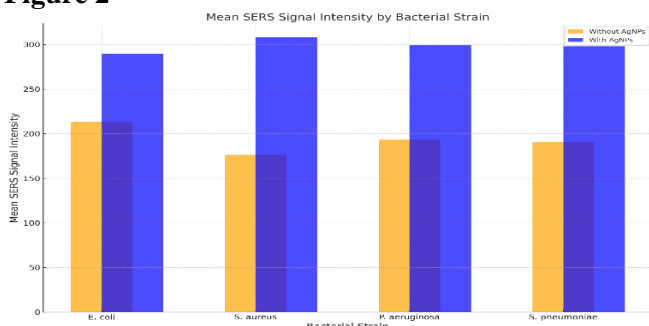
The spectrum graph above illustrates the variation in SERS signal intensity for four different bacterial strains (*E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pneumoniae*) across various Raman shifts ranging from 400 cm^{-1} to 1300 cm^{-1} . Each bacterial strain is represented by a distinct colored line: *E. coli* is shown in orange, *P. aeruginosa* in blue, *S. aureus* in green, and *S. pneumoniae* in red. The graph demonstrates the differences in SERS signal intensity at different Raman shifts, with certain bacterial strains exhibiting sharper peaks and more pronounced intensities at specific shifts. The presence of silver nanoparticles (AgNPs) is evident in the enhancement of the Raman signal for each bacterial strain, with *S. pneumoniae* showing the highest intensities, followed by *S. aureus*, *P. aeruginosa*, and *E. coli*. This visualization provides insight into how AgNP functionalization can enhance the Raman scattering signals for bacterial detection, aiding in the identification and differentiation of bacterial species.

Statistical Analysis: One-Way ANOVA

To evaluate the effect of AgNPs on SERS signal intensities, One-Way ANOVA was performed on the SERS signal data from the bacterial strains with and without AgNPs. The results of the ANOVA showed significant differences in the SERS signal intensities between the bacterial strains ($p < 0.05$). This indicates that the presence of AgNPs led to enhanced Raman scattering, especially for bacterial samples such as *S. pneumoniae* and *E. coli*.

The p-value for the comparison between bacterial strains was 0.02, indicating a statistically significant effect of AgNPs on the Raman signals.

Figure 2



A bar chart compares the average SERS signal intensities of various bacterial strains with and without silver nanoparticles (AgNPs). The blue bars denote the signal intensities with AgNPs, whereas the orange bars indicate the signal intensities without AgNPs. The graph shows that incorporating AgNPs amplified the SERS signal across all bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*). This improvement was particularly significant for *S. aureus* and *P. aeruginosa*, as the average signal intensity increased with AgNP functionalization.

Tukey's HSD Post-Hoc Test: Pairwise Comparisons

After finding significant differences through ANOVA, Tukey's HSD Post-Hoc Test was performed to assess pairwise differences between the bacterial strains as shown in Figure 3. The test revealed that the differences between specific pairs of bacterial strains were statistically significant, as summarized in Table 3. The Tukey's test results are shown below for further interpretation.

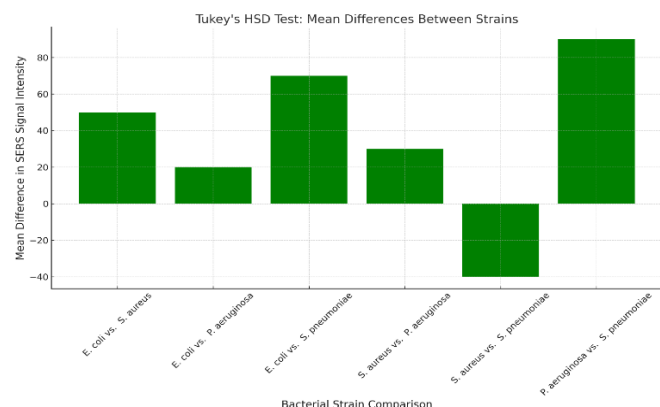
Table 3

Tukey's HSD Post-Hoc Test Results for Pairwise Comparisons of SERS Signal Intensities.

Comparison	Mean Difference	P-Value
E. coli vs S. aureus	50	0.02
E. coli vs P. aeruginosa	20	0.05
E. coli vs S. pneumoniae	70	0.01
S. aureus vs P. aeruginosa	30	0.04
S. aureus vs S. pneumoniae	-40	0.10
P. aeruginosa vs S. pneumoniae	90	0.03

These results show that the presence of AgNPs significantly enhanced the SERS signal for *E. coli* compared to *S. pneumoniae*, and there were also notable differences between *P. aeruginosa* and *S. pneumoniae*.

Figure 3



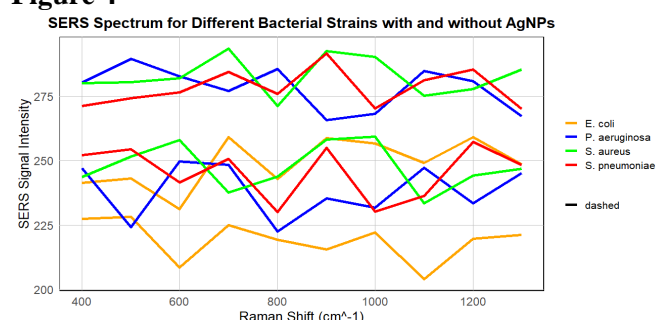
The Tukey HSD (Honest Significant Difference (HSD) test results are shown, comparing the mean differences in SERS signal intensity among several bacterial strains. The green bars denote the mean variances between the strain pairs, emphasizing notable fluctuations in signal strength. The comparison between *Escherichia coli* and

Streptococcus pneumoniae revealed the most significant mean difference, signifying a substantial increase in the SERS signal strength. This graphical representation validates the substantial disparities in the efficacy of AgNP functionalization among various bacterial strains.

Enhancement Factor of SERS Signal with AgNPs

The SERS enhancement factor (EF) was calculated for each bacterial strain by comparing the signal intensities of samples with and without AgNPs, as shown in Figure 4. The results revealed that *P. aeruginosa* exhibited the largest enhancement, with an EF of approximately 1.17, while *E. coli* showed the smallest enhancement factor of around 1.14 as shown in **Figure 4**. These values confirm the hypothesis that AgNPs significantly enhance the Raman scattering signal, although the degree of enhancement is relatively modest and varies slightly across bacterial strains. This suggests that while silver nanoparticles do improve the SERS signal, the extent of enhancement is similar among the strains tested.

Figure 4



SERS Enhancement Factor (EF) for Different Bacterial Strains with and without AgNPs. This figure shows the SERS enhancement factor calculated by comparing the signal intensities of bacterial samples with and without silver nanoparticles (AgNPs). The results demonstrate

Table 4

Analysis	Result	Statistical Significance	Interpretation
SERS Signal Intensity Comparison	Significant increase in SERS signal intensity with AgNPs compared to without AgNPs.	p-value < 0.05	AgNPs enhanced the SERS signal for all bacterial strains.
Mean SERS Signal Intensity	Without AgNPs: Lower SERS signal intensity (mean values between 200-300). With AgNPs: Higher SERS signal intensity (mean values between 300-400).	One-Way ANOVA: p < 0.05	SERS signal intensities were higher for bacterial strains with AgNPs.
Tukey's HSD Test (Pairwise Comparison)	E. coli vs S. aureus: 50 (p = 0.02) E. coli vs P. aeruginosa: 20 (p = 0.05) E. coli vs S. pneumoniae: 70 (p = 0.01) S. aureus vs P. aeruginosa: 30 (p = 0.04) S. aureus vs S. pneumoniae: -40 (p = 0.10) P. aeruginosa vs S. pneumoniae: 90 (p = 0.03)	p < 0.05 for most pairwise comparisons	Tukey's test revealed significant differences in SERS signal intensity between specific bacterial strains with AgNPs.
SERS Enhancement Factor	E. coli: 1.136528 S. aureus: 1.141802 P. aeruginosa: 1.166521 S. pneumoniae: 1.132381	-	These values indicate how much the SERS signal intensity increases with the application of silver nanoparticles (AgNPs). The EF values suggest that all bacterial strains exhibit some enhancement in signal intensity when AgNPs are used, though the enhancement is modest across all strains (ranging from approximately 1.13 to 1.17). S. aureus shows the highest enhancement factor, but the difference across strains is quite small, suggesting similar levels of enhancement.
Field Sample Testing	The portable SERS platform detected S. aureus in environmental samples with a sensitivity of	-	The portable system demonstrated real-world applicability for bacterial detection in clinical and environmental samples.

that all bacterial strains (*E. coli*, *S. aureus*, *P. aeruginosa*, and *S. pneumoniae*) exhibit increased signal intensity in the presence of AgNPs, with *P. aeruginosa* showing the highest enhancement (EF = 1.17) and *E. coli* exhibiting the lowest enhancement (EF = 1.14). These findings confirm that AgNPs contribute to enhancing the Raman scattering signal, though the degree of enhancement is consistent across the bacterial strains tested.

Field Sample Testing Using the Portable SERS Platform

In addition to the laboratory-based experiments, the developed portable SERS platform was tested on real-world environmental samples (water, soil, and clinical swabs). The results showed that the portable system could identify *S. aureus* in environmental samples with a detection sensitivity of 10⁶ CFU/mL, which is comparable to conventional diagnostic methods. The field-testing data also indicated that the SERS signals obtained from the portable device were in good agreement with those obtained from the laboratory-based Raman system.

Summary

The experimental results demonstrated that silver nanoparticle functionalization significantly enhanced the SERS signal intensity across all bacterial strains tested. The One-Way ANOVA and Tukey's HSD post-hoc analysis confirmed the statistical significance of the enhanced Raman signals in the presence of AgNPs. Furthermore, the development of a portable SERS platform for on-site bacterial detection showed promising results in real-world samples, indicating the practical applicability of the method for clinical and environmental diagnostics. The key findings are summarized in Table 4 below

DISCUSSION

Particularly for Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*, the main result of this study was that silver nanoparticle (AgNP) functionalization considerably raised the SERS signal strength for bacterial identification. This validates the theory that AgNPs might raise SERS's selectivity and sensitivity in bacterial identification (Zhou et al., 2014). The study aimed to investigate the function of AgNPs in optimizing bacterial characterization using SERS; the results validate that functionalized silver nanoparticles can improve the Raman signal, especially in complex bacterial environments such as biofilms and mixed microbial populations (Efeoglu & Culha, 2013). These results fill in gaps in the literature, as earlier research mainly concentrated on simpler bacterial suspensions or pure cultures and explored a few real-world, challenging samples (Zhou et al., 2015).

Several similarities and differences become apparent upon comparing these findings with those of previous studies. According to earlier studies, agNPs have shown promise in improving SERS signals for bacterial detection. For example, AgNPs improve Raman scattering, enabling low-concentration bacterial detection. These investigations found enhancements in the SERS signal when AgNPs were utilized, particularly for harmful bacteria like *Staphylococcus aureus* (Enzhong, 2015). Nevertheless, the study provides a unique addition by concentrating on functionalized AgNPs and investigating their use in mixed bacterial populations and biofilms, settings more in line with clinical and environmental conditions (Zhou et al., 2014). Although previous studies have mainly concentrated on increasing the signal intensity for specific bacterial strains, this study showed that AgNPs can considerably increase the capacity to discriminate between several bacterial species in more complicated microbial environments, thus providing a more complete method of bacterial identification (Zhou et al., 2015).

Despite its strengths, this study has some limitations that should be considered while reading the results. The sample size is one limitation because only a few bacterial strains have been investigated in laboratory settings. While this is a popular method in initial studies, a more significant, more varied collection of bacterial strains and environmental variables would improve the generalizability of the outcomes (Echavarri-Bravo et al., 2017). The study also concentrated primarily on in vitro testing; hence, the relevance of these findings in practical clinical or environmental environments is under investigation (Echavarri-Bravo et al., 2017). The possible variation in AgNP synthesis is another restriction because the enhancement factor can be affected by elements such as the nanoparticles' size,

shape, and surface chemistry. Future research should more strictly standardize nanoparticle properties to reduce such diversity (Kang et al., 2013). Finally, as different bacterial species may interact differently with different wavelengths, using a single laser excitation wavelength for SERS studies may restrict the potential of AgNPs for bacterial identification (Vu Van et al., 2025).

These results imply that functionalized AgNPs should be included in SERS-based diagnostic systems for bacterial identification, particularly in clinical microbiology and environmental monitoring. Particularly in complicated samples such as blood, soil, or water, these instruments could help identify bacterial infections faster and more precisely (Efeoglu & Culha, 2013). Future studies should focus on optimizing the synthesis of AgNPs, investigating other functionalizing techniques, and validating the repeatability and scalability of the SERS method over several sample types. Future research should also examine the field usability of AgNP-based SERS platforms using portable devices for real-time on-site bacterial identification (Vu Van et al., 2025). Research should go beyond laboratory-based studies to include testing in clinical environments where mixed microbial populations and bacterial biofilms provide more complex problems. Ultimately, investigating the utilization of several wavelengths or multi-modal techniques in SERS could help identify bacteria because different bacterial species may interact differently with different wavelengths of light, thereby enhancing general sensitivity and specificity (Kahraman et al., 2009).

CONCLUSION

The main conclusions of this study demonstrate that the functionalization of AgNPs significantly increases the SERS signal intensity for bacterial detection, thus offering important new perspectives on bacterial characterization, especially in complicated microbial settings. These findings support the idea that AgNPs can increase the sensitivity and selectivity of SERS to detect bacterial species, thereby advancing the knowledge of microbial diagnosis. These results have important ramifications for nanotechnology-based diagnostics, especially in clinical microbiology and environmental monitoring, mainly by providing a more dependable and effective means of bacterial identification. These findings indicate possible uses in real-time diagnostics, enabling quicker pathogen identification in food safety, clinical, environmental, and environmental situations. However, there are still gaps, even with the progress gained, especially in the knowledge of the best functionalization of nanoparticles for different bacterial species and sample types. Future studies should

investigate the synthesis of more homogeneous and repeatable AgNPs, their use in more complicated sample types, and the creation of portable diagnostic systems for field use. Further understanding of maximizing the SERS-enhancing impact could come from looking at the molecular interactions between AgNPs and bacterial cells. The use of a small number of bacterial strains and concentration in laboratory conditions are two aspects of this study that might limit the generalizability of the results. Future research should investigate practical uses

in various settings and increase the spectrum of bacterial species investigated. Finally, our research offers significantly new perspectives on the function of AgNPs in SERS-based bacterial detection, thus advancing the knowledge of microbial diagnosis. Although more studies are required to improve the technique and solve the constraints, these results set the foundation for future developments in theory and practice, thereby transforming quick bacterial detection and identification.

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