J Nanostruct 16(3): 4108-4123, Summer 2026

RESEARCH PAPER

Raoultella Spp. Catalysed Biosynthesis of Ag NPs: Isolation, Characterization Optimization and Bio Application

Hussein Hameed Al-Turnachy

Department of Biology, Faculty of Science, University of Kufa, Najaf, Iraq

ARTICLE INFO

Article History:
Received 02 March 2026
Accepted 24 June 2026
Published 01 July 2026

Keywords:
Green synthesis
Raoultella spp.
AgNPs Optimization and Characterization
Silver rich soil
rpoB sequencing

ABSTRACT

Finding heavy-metal-tolerant microflora in silver-enriched soils offers a truly sustainable foundation for green nanomanufacturing. For this study, various silver-resistant bacterial strains were evaluated optimal potent isolate for biosynthesis of silver nanoparticles. *Raoultella ssp* isolate performed exceptional potency. The Isolate was identified by combining automated VITEK-II compact system technology, Phenotypic profiling, Biochemical reactions, *rpoB* gene sequencing, and profiling. To drive the extracellular reduction of silver nitrate into silver ion, cell-free supernatants harvested from Brain Heart Infusion broth cultures of *Raoultella spp.* By optimizing the reaction conditions, its found that the ideal synthesis conditions were a pH of 8.0, a temperature of 37°C, and an 18-hours incubation. Furthermore, the concentration of the precursor substrate directly controlled the attributes of the nanoparticles. A comprehensive morphological and elemental analysis was evaluated by Scanning electron microscopy, energy-dispersive X-ray spectroscopy, atomic force microscopy, and X-ray diffraction. The findings confirmed that synthesized silver nanoparticles was monodispersed, spherical and homogenous. These particles had a mean diameter of 21.9 nm. Finally, our biological assays revealed that these particles possess strong antibacterial, antibiofilm, and antioxidant properties This highlights the clear therapeutic potential of these specific biogenic nanomaterials.

How to cite this article
Al-Turnachy H., Dorkoosh F. Raoultella Spp. Catalysed Biosynthesis of Ag NPs: Isolation, Characterization Optimization and Bio Application. J Nanostruct, 2026; 16(3):4108-4123. DOI: 10.22052/JNS.2026.03.094

INTRODUCTION

Nanobiotechnology positioned right at the intercept of materials science and biology It provides entirely new ways to approach sustainable manufacturing. Traditional chemical and physical methods for fabrication of nanoparticles usually require massive amounts of energy They also rely on hazardous reagents and generate toxic byproducts. Because of this, biogenic synthesis has become a highly attractive green alternative [1]. This approach uses natural metabolic pathways to create biocompatible, water-dispersible nanomaterials right at room temperature Microorganisms are especially useful here They have the natural enzymatic machinery needed to control the nucleation and growth of metallic nanostructures, keeping their dimensions highly specific Soil environments act as massive

* *Corresponding Author Email: husseinh.abdulla@uokufa.edu.iq*

microbial reservoirs They support complex communities of microbes that have adapted to a wide range of environmental stressors [2]. When exposed to heavy metals, certain specialized strains are forced to evolve strong detoxifying and resistance pathways just to survive. This includes surviving exposure to toxic ionic silver [3]. Members of the genus Raoultella fall into this resilient category and show remarkable metabolic diversity They carry the genetic traits for silver resistance, but they also maintain active functional pathways for nitrate reduction and nitrogen fixation [4]. The construction of efficient bio-factories for nanoparticle production requires, as a necessary first step, the isolation of highly specialized strains from metal-rich habitats [5]. How well a nanomaterial functions depends entirely on its physical traits This includes its geometry, size distribution, dispersion quality, and surface morphology. To get precise control over these structural features, researchers have to carefully adjust specific operational parameters during the synthesis phase Factors like incubation time, reaction pH, temperature, and the concentration of precursor ions play a massive role They directly dictate the nucleation kinetics and the final dimensions of the particles Silver nanoparticles have broad-spectrum biological activity As a result, they remain a massive focal point across optical, electronic, and biomedical research fields [6] Using microbial extracellular production to fabricate AgNPs is both scalable and highly eco-friendly These specific biogenic AgNPs show incredible promise as new antibiofilm and antimicrobial agents, specifically engineered to fight multidrug-resistant pathogens. Building on this, our investigation focuses on optimizing both the biosynthesis and characterization of silver nanoparticles fabricated by *Raoultella spp.* isolated directly from a silver-rich environment, and the antimicrobial efficacy of the resulting silver nanoparticles ultimately evaluated [7,8].

MATERIALS AND METHODS

Preparation of cell free supernatant of Raoultella spp.

Raoultella spp. Was identified through a comprehensive screening process involving numerous bacterial isolates from soil samples, primarily due to Its notable resistance to commercial silver nanoparticles. (AgNPs) and its potential for extracellular AgNP biosynthesis. The selected *Raoultella spp*. Was inoculated into Brain Heart Infusion (BHI) broth and subsequently incubated under aerobic conditions at 37°C for a period of 24 hours. Identification of *Raoultella spp.* colonies was confirmed through morphological and biochemical analyses utilizing VITEK, in addition to *rpoB* gene sequencing, as detailed by [9]. To prepare the cell-free supernatant (CFS) from the Raoultella spp. culture, the broth culture underwent centrifugation at 4500xg for 20 minutes at 4°C. This cell-free supernatant was then employed for the biosynthesis of silver nanoparticles, a methodology consistent with the findings of [10].

Molecular identification of Raoultella spp.

Genomic DNA was extracted from Raoultella spp. Using the G-spin DNA extraction kit (Cat #17045 intron biotechnology/Korea), in accordance with the manufacturer's instructions. DNA concentration was quantified spectrophotometrically by measuring the optical density at 260 nm. DNA purity was ascertained through the OD260/OD280 ratio, with a range of 1.8±0.2 signifying pure DNA, as cited by [11].

The PCR assay was conducted to identify Raoultella species utilizing universal primers targeting the *rpoB* gene, specifically the Forward Primer (*rpoB*-F / Vicp1):

5'- CAG GTC GTC ACG GTA ACA AG -3'

Reverse Primer (*rpoB*-R / Vicp2):

5'- GTG GTT CAG TTT CAG CAT GTA C -3'

The (512bp) PCR product was electrophoresed on a 1% agarose gel, stained with RedSafe, and documented using a UV gel imaging system.

Gel extraction Protocol for (Sequencing)

Right before extraction, reconstituted the wash reagent by mixing in absolute ethanol. To isolate the DNA from the agarose matrices, the protocol previously established by [12] was strictly followed.

Sequencing and Sequence Alignment

The PCR products were immigrated using 2% agarose gel. After staining the gel with Red-Safe and documented the results at a wavelength of 302 nm. Then sent the PCR product out for sequencing at the National Instrumentation Center for Environmental Management (NICEM), where they utilized an Applied Biosystems DNA Sequencer 3730XL. For homology searches, BLAST

tool available through the NCBI platform applied. Finally, multiple alignments was assessed using the BioEdit software program.

Biosynthesis of AgNPs using cell free supernatant

Metabolic byproducts produced by *Raoultella spp.*Drove the entire extracellular synthesis of silver nanoparticles. silver nitrate was used as a precursor metallic salt. To figure out the absolute ideal precursor molarity, different concentrations (1, 2.5, 5, 7.5, and 10 mg/ml) of AgNO3 inoculated independently straight into the bacterial cell-free supernatant with agitation of samples to guarantee complete homogenization. Silver ions are prone to light-induced oxidation, so every single mixture were kept in strict dark conditions.

To optimize the PH of reaction mix, a range of reaction PH values was calibrated to specific pH levels (5, 6, 7, 8, and 9) and ran them alongside a designated control group and incubated at 37°C for 24 hours, keeping them under continuous agitation at 150 rpm.

Temperature optimization includes incubation of the reaction mix at broad thermal gradient ranging from 22°C up to 50°C. During thermal optimization, agitation maintained at robust 150 rpm. Once nailed down the absolute optimal kinetic parameters, bulk biosynthesis scaled up to a 1-liter vessel You could actually see the bio reduction of silver nitrate into AgNPs happening in real time. The broth transformed from a very pale yellow to light brown hue into a stark, deep brown When the reaction finished, AgNPs pelleted the particle suspensions using ultra-centrifugation at 10,000 rpm for 25 minutes, the exhausted supernatant dumped and the remaining nano-pellets were passed through a strict purification cascade. This involved washing them five consecutive times using sterile distilled water, followed by final wash in ethanol just to strip away any unreacted organic residues left behind. Next, the purified AgNP yield was taken and thermally dehydrated in a drying oven set to 50°C for roughly 18 to 24 hours, final dried nano-powder was collected meticulously and to undergo all of the downstream structural and functional evaluations [13].

Characterization of Biogenic silver nanoparticles
XRD analysis

The Department of Geology at the Faculty of Science, Baghdad University, handled the X-ray diffraction characterization for our silver nanoparticles.

SEM analysis

To achieve both the geometric features and the surface morphology of the biogenic nanomaterials, Scanning Electron Microscope (SEM) analysis utilized using (Inspect S50 FEI Scanning Electron Microscope) at the Faculty of Science's Electron Microscopy Unit / University of Kufa, operational settings has been configured to exactly match the parameters described by [14] Specifically, at 15 kV accelerating voltage under low-vacuum conditions, set the spot size to 4, and maintained a focal working distance somewhere between 5 and 10 mm.

EDS analysis

To get the stoichiometric profiling and elemental mapping of the nanoparticles, Bruker EDS module attached to the SEM apparatus at the University of Kufa mentioned earlier was used. It's essential to made sure to acquire all point analysis spectra under perfectly standardized conditions and locked the accelerating voltage at 10 kV, kept the spot size at 5, and held a constant working distance of 10 mm to guarantee our compositional mapping was as precise as possible [15].

AFM analysis

Mean particle dimensions, spatial distribution, three-dimensional geometric characteristics, and overall surface topography using an Atomic Force Microscope (Model: CSPM-AA3000-220V, Angstrom Advanced Inc. (USA). At the Department of Chemistry at the Faculty of Science, Baghdad University, hosted this specific analytical phase of the study.

Antibacterial activity of Silver Nanoparticles

Antimicrobial potential the Raoultella-derived AgNPs were investigated against a broad panel of clinically relevant Gram-positive and Gram-negative pathogens after optimization of AgNPs concentration by screaning of a gradient dilution (0.37, 0.75, 1.5, 3, 4.5, 6 mg/ml) against (staphylococcus aureus and E. coli), a standard agar well diffusion protocols were applied for this step [16]. The inocula carefully standardized so they would match a 0.5 McFarland turbidity standard, which comes out to roughly 1.5 × 10^8 CFU/mL microbial suspensions and uniformly spread each of the two sample bacteria across freshly poured

Brain Heart Agar plates then bored using a sterile cork borer equidistant 6 mm cylindrical wells right into the inoculated agar. An amount of 100 µL each concentration aliquots was dispensed into each well. Similarly, other plates set were inoculated with clinically significant isolates and bored each double well. One well inoculated with biogenic AgNPs, the other well inoculated with commercial AgNPs. The optimal (1.5mg) concentration of biosynthetic AgNPs, while the other received same concentration (1.5mg/ml) of a commercial AgNP as a control. The plates statically incubate at 37°C After 24 hours, every plate visually inspected and measured the resulting zones of inhibition around each well and quantified them in millimeters [17].

Antibiofilm activity of silver nanoparticles

Microtiter plate assays have been utilized to evaluate Antibiofilm potential of the biogenic AgNPs. Both biogenic and commercial AgNP was suspended and diluted independently down to three final working concentrations: 100, 150, and 200 µg/mL First, bacterial inocula was adjusted until they hit an optical density OD 600 (0.5 McFarland turbidity standard). A 0.1 mL volume of this standardized suspension then inoculated straight into 1.9 mL of Tryptic Soy Broth (TSB). From that seeded broth, 150 µL dispensed into the individual wells of a sterile 96-well microplate. Immediately after that, 50 µL of the different AgNP treatments was added into the wells. To mitigate medium evaporation, all the outermost perimeter wells were filled with 200 µL of sterile deionized water. Finally, the microtiter plates were incubated at 37°C for 16 hours Once the full incubation cycle, wrapped up and gently decanted all the unattached planktonic cells, then fixed the adherent biofilm structures in place using 99% methanol. After they were properly fixed, microtiter plates have been rinsed twice using sterile saline and let them completely air dry. To quantify the biomass, followed by addition of 200 µL of a 0.2% crystal violet stain to every well and let it sit for exactly 5 minutes. Whatever excess dye remained was discarded and washed the plates two more times to flush out any unbound stain, and waited for the microplate to completely dry again. Once it was ready, 33% acetic acid introduced to resolubilize the entrapped crystal violet dye. Finally, the microplate was read the absorbance at 570 nm on a microplate spectrophotometer to quantify both biofilm and biomass in the biofilm. This entire analytical framework was adapted from methods described by [18].

Antioxidant activity of biogeneic silver nanoparticles in vitro

1, 1-diphenyl-2-picrylhydrazyl free radical

Fig. 1. Macroscopic illustration of *Raoultelas p.* isolate, the figure shows colonial morphology and cultural characteristics.

scavenging assay was utilized to evaluate the capacity of both commercial and biogenic AgNPs, synthesized by Raoultella spp., to neutralize DPPH free radicals This assessment followed a modified protocol based on [19]. A DPPH solution exhibiting an absorbance of 0.98±0.02 served as the control, with methanol employed as the blank. Biogenic AgNPs derived from Raoultella spp. And commercial AgNPs were tested at concentrations of 0.75, 1.5, and 2 mg/ml in methanol. Each concentration of biogenic AgNPs was individually combined with 3 ml of the DPPH working solution; this procedure was replicated for all three concentrations. Commercial AgNPs were similarly prepared with DPPH. The reaction mixtures were incubated for 30 minutes in a dark environment at 37°C, and the absorbance (A) of the test samples (T) was measured at 517 nm using a spectrophotometer. This experiment was conducted in triplicate. The inhibition of the DPPH radical by biogenic AgNPs was determined using the formula provided by [20] and [21]:

$$\% \text{ Antioxidant} = ([(AbsC - AbsB) - (AbsT - AbsB)]/(AbsC - AbsC)) \times 100$$

RESULTS AND DISCUSSION

Macroscopic identification

The colonies present as small, smooth, and viscous, accompanied by an unpleasant odor. Through successive sub-culturing, Raoultella spp. Consistently maintains its characteristic slimy and smooth colonial morphology. (Fig. 1).

Biochemical identification

Biochemical identification of *Raoultella sp,* when compared to Klebsiella sp, reveals a high degree of similarity, often leading to misidentification as Klebsiella sp. Even with 16S rRNA sequencing, researchers rely on *rpoB* gene sequencing as the gold standard method for accurate differentiation.

Biochemical characterization of *Raoultella spp*. Demonstrates distinct phenotypic differences from Klebsiella spp., specifically indole negativity, positive ornithine decarboxylase activity, and growth at 10 °C but not at 44.5 °C. These characteristics underscore how these organisms adapt to specialized ecological niches while offering phenotypic criteria for taxonomic separation Although conventional biochemical profiling provides preliminary diagnostic value, high-resolution techniques‹specifically *rpoB* locus sequencing and MALDI-TOF mass spectrometry‹have proven indispensable for definitive species resolution The clinical urgency of accurate identification is further elevated by the global emergence of carbapenemase-producing Raoultella phenotypes carrying resistance determinants like blaNDM-1 and blaKPC-2 These cumulative data reinforce the classification of Raoultella as a distinct generic clade, despite overlapping metabolic signatures and variable carbohydrate fermentation traits that compromise the reliability of single-marker phenotypic assays Consequently, combining full-genome sequencing with phenotypic characterization remains critical for advancing diagnostic precision and uncovering the broader epidemiological and ecological dynamics of Raoultella species [22].

Identification using VITEK2

VITEK-II compact automated system has been used to run an initial phenotypic screening of the *Raoultella spp.*. This process utilized a Gram-Negative Identification card that comes pre-loaded with 64 distinct biochemical assays After a 4-hour analysis window, the system spit out a phenotypic profile It identified the sample as Klebsiella

Table 1. Biochemical reactions of *Raoultella spp.* Compared with *Klebsiella spp*.

Biochemical test	Bacteria			
	R. terrigena	*R. electrica*	*K. pneumoniae*	*K. oxytoca*
Indole	-	-	-	+
Ornithine decarboxylase (ODC)	-	-	-	-
Lysine decarboxylase (LDC)	+	+	+	+
Melezitose / Sorbose Fermentation	+	Variable	Compatible	Compatible
Growth at 10 °C	+	+	-	-
Growth at 44.5 °C	-	-	+	+

pneumoniae with a 93% confidence probability.

Molecular identification

Because closely related bacterial species may exhibit similar biochemical profiles, biochemical identification alone may result in misidentification. Therefore, molecular identification was performed to confirm the bacterial identity. Genomic DNA was first extracted from the bacterial isolates, followed by amplification and sequencing of the *rpoB* gene. The obtained sequences were then used to establish the precise genotypic identity of the isolates and provide molecular confirmation of the phenotypic identification.

Amplification of rpoB gene

Genomic DNA templates, extracted and validated through agarose gel electrophoresis, were employed for the amplification of the *rpoB* gene. This amplification was performed using universal *rpoB* gene primers, following the protocol detailed in (Table 2) and duly documented. The resulting 512 bp *rpoB* DNA bands, as depicted in (Fig. 2), were subsequently extracted for sequencing.

Sequencing the rpoB gene

The *rpoB* gene sequencing results were obtained online and subsequently aligned with the NCBI database utilizing Blast software. Phylogenetic analysis identified the isolate as *Raoultella spp.* (Fig. 3).

Pairwise alignment

A pairwise alignment and distance phylogeny analysis of bacterial *rpoB* gene sequences was

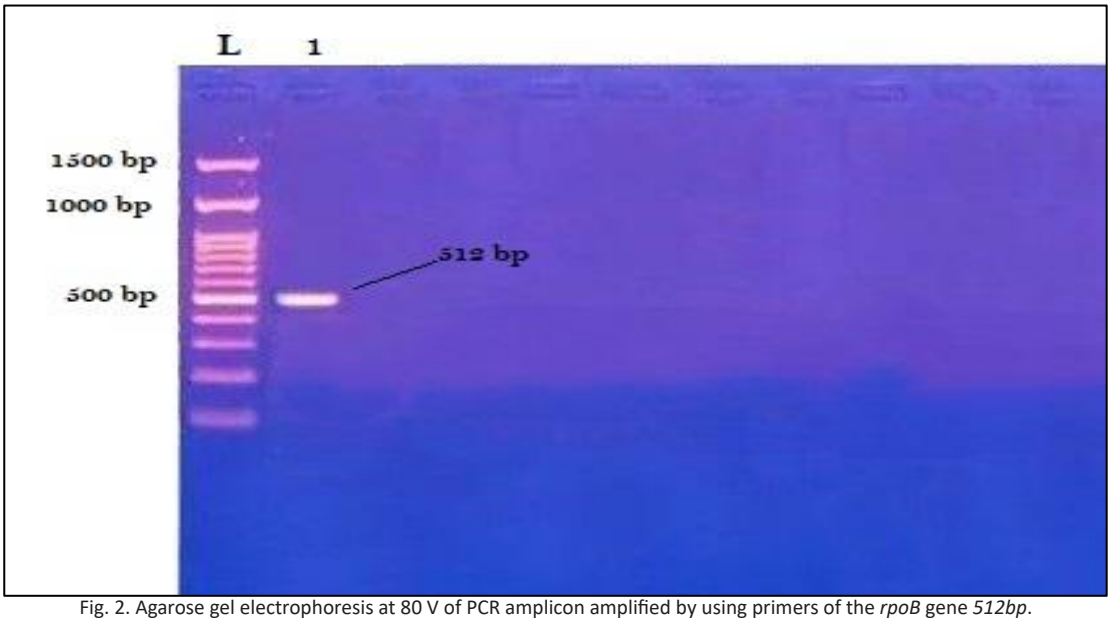

Fig. 2. Agarose gel electrophoresis at 80 V of PCR amplicon amplified by using primers of the *rpoB* gene *512bp*.

Table 2. The optimum PCR conditions applied in PCR reaction for *rpoB* gene.

No.	Phase	T (°C)	Time	No. of cycle
1	Initial Denaturation	95 °C	3 min	
2	Denaturation -2	95 °C	45sec	
3	Annealing	52 °C	45sec	40 cycles
4	Extension-1	72 °C	50sec	
5	Extension -2	72 °C	10 min	

conducted using BLAST online. The closest genetic relative to *Raoultella terrigena* (EU623235) was identified with 98% identity and an E-value of 0.0.

Optimization of synthesis conditions

Prior to the large-scale production of biogenic silver nanoparticles (AgNPs), the environmental parameters, including pH, substrate concentration, temperature, and reaction time, underwent a comprehensive optimization process.

pH optimization

The most effective Bio-Nano-Synthesis activity, evidenced by the antimicrobial efficacy of biogenic AgNPs as quantified by inhibition zone diameter in millimeters and discernible color transformation, was identified at pH within the range of five discrete levels (5, 6, 7, 8, and 9). (Table 3).

Optimization of substrate concentration

The optimization of silver nitrate (AgNO3) substrate concentration was conducted utilizing five distinct concentrations (1, 2.5, 5, 7.5, and 10 mg/ml) for the nano-biosynthesis process involving Raoultella sp. The results indicate a direct correlation between productivity and

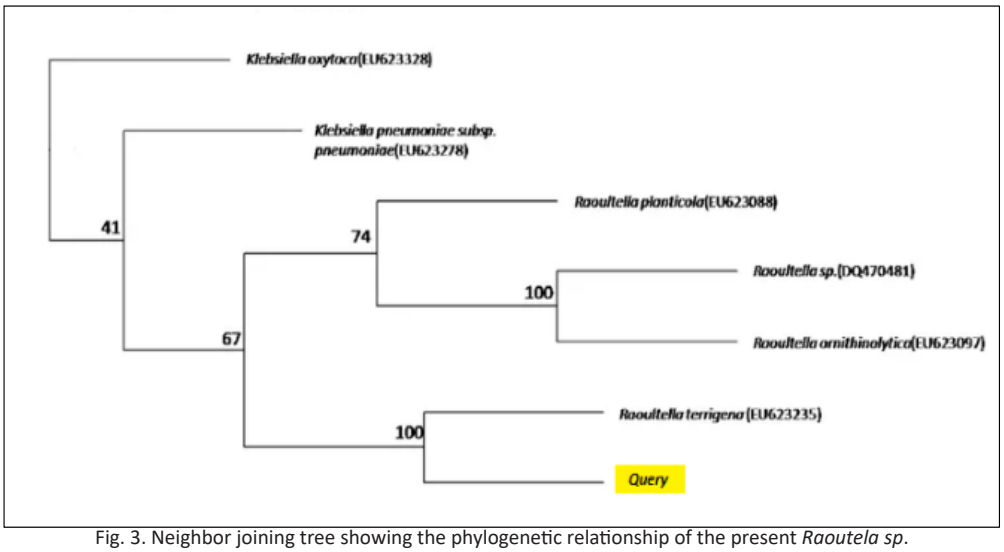

Fig. 3. Neighbor joining tree showing the phylogenetic relationship of the present *Raoutela sp*.

Table 3. Inhibition zones produced by AgNPs fabricated at different pH values.

pH	Inhibition zone in (mm) against pseudomonas
5	20
6	26
7	24
8	27
9	19

Table 4. The inhibition zone generated by extracellularly produced silver nanoparticles (AgNPs) from Raoultella species was evaluated across various silver nitrate ($AgNO_3$) concentrations.

Isolate	Inhibition zone in (mm) produced by substrate concentration				
	1 mM	2.5 mM	5 mM	7.5 mM	10 mM
Raoultella spp..	11	15	17	17	21

substrateconcentration. (Table 4).

Optimization of incubation temperature

Thermal parameters needed to figure out exactly the optimal temperature would yield the absolute maximum extracellular yield of silver nanoparticles. the biogenic reaction was taken and ran it through a sweeping temperature gradient from 22°C up to 52°C. Specifically, batches of thermal parameters were examined at 22, 27, 32, 37, 42, 47, and 52°C (Table 5). When these kinetic batches analyzed, the data showed a very clear spike in biosynthetic efficiency right at 37°C. in order to validate optimum temperature, two different primary indicators First, the visual intensity of the macroscopic color shift, which signals nanoparticle nucleation Second, the bactericidal potency of that specific colloidal suspension examined against a reference bacterial Isolate Both indicators confirmed 37°C was the sweet spot.

Production of AgNPs

The metabolic byproducts floating in the cell-free supernatant of *Raoultella spp* Turned out to be incredibly effective at driving the extracellular bioreduction of silver ion into nanosilver. the nanomanufacturing sequence has been kicked off by introducing our precursor metal salt into the sterile broth, making sure to use the optimal parameters had just established. The mixture underwent continuous orbital agitation at 200 rpm During this time; the reaction vessel must be monitored for any distinct optical changes to confirm that the ionic silver was actually converting into stable AgNPs. and documented a very consistent chromogenic transition. The liquid started off as a pale yellow to light broun, but it progressively darkened until it became a deep, rich reddish-brown suspension This macroscopic shift is actually a highly reliable visual hallmark It definitively confirms the localized surface plasmon resonance of the newly formed biogenic nanoparticles. (Fig. 4).

Characterization of biogenic AgNPs

Antimicrobial activity

The antibacterial efficacy of biogenic silver

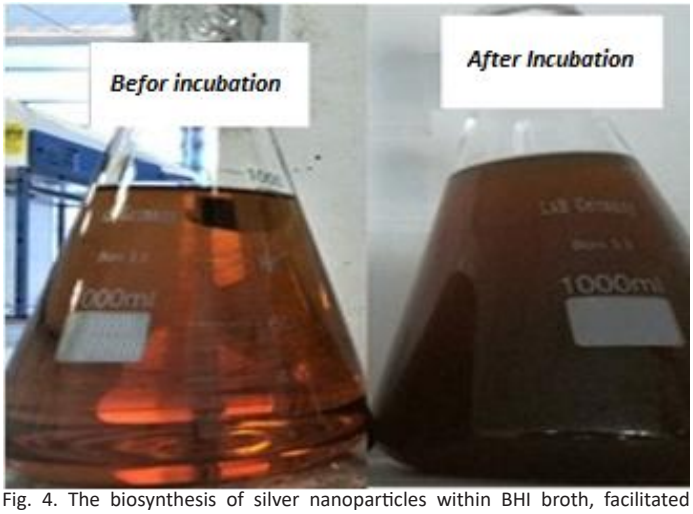

Fig. 4. The biosynthesis of silver nanoparticles within BHI broth, facilitated by Raoultella spp., resulted in a distinct color transformation from an initial yellowish hue to a reddish-brown coloration following the synthesis process.

Table 5. Optimization of incubation temperature applied for synthesis of AgNPs by *Raoultella spp.* indicated by inhibition zone measured in (mm).

Strain	Inhibition zones at						
	22 °C	27 °C	32 °C	37 °C	42 °C	47 °C	52 °C
Raoultella spp.	17	14	15	20	17	17	-ive

nanoparticles (AgNPs) is considered a benchmark. Thus the antimicrobial activity of biogenic AgNPs synthesized by *Raoultella spp. Was* evaluated. The agar well diffusion method was employed to evaluate efficacy against a range of clinical bacteria. Commercial silver nanoparticles were utilized as a control in this investigation.

The findings It has been observed that at a concentration of 150 µg/ml, Gram-positive bacteria demonstrated the most significant zone of inhibition. in *Staphylococcus aureus* (18mm) when compared to commercial AgNPs. Notably, commercial AgNPs were resisted by all tested AgNPs against the standard bacterium *Pseudomonas sp.* Furthermore, the results demonstrated optimal production at 37°C, with a diminished activity observed at 52°C for the production. (Table 6).

Among the gram-negative bacteria investigated, AgNPs produced by *Raoultella spp.* Demonstrated the largest zone of inhibition against Pseudomonas aeruginosa (31mm), followed by *Klebsiella spp.* (24mm) and E. coli (23mm). Overall, gram-negative bacteria exhibited greater sensitivity to both biogenic and commercial AgNPs compared to gram-positive bacteria, with biogenic AgNPs displaying superior activity (Table 6).

Optimization of time of production

The optimal incubation period for extracellular silver nanoparticle (AgNP) biosynthesis was determined to be 10 hours, with sampling conducted every 4 hours (Table 7).

Antioxidant activity

The radical scavenging capacity of the biogenic silver nanoparticles synthesized via Raoultella spp was assessed employing a standard 2,2-diphenyl-1-picrylhydrazyl colorimetric assay In this analytical framework, effective neutralization of free radicals typically corresponds to a measurable decrease in spectrophotometric absorbance at 517 nm Intriguingly, empirical measurements revealed an unexpected inverse trend: the introduction of the biogenic AgNPs resulted in an absorbance reading higher than that of the untreated control This

Table 6. evaluation of the antimicrobial efficacy of silver nanoparticles (AgNPs) synthesized using *Klebsiella pneumoniae*, as well as commercially available AgNPs, against various pathogenic bacteria. This evaluation was conducted at a concentration of 150 µg/ml and measured by the diameter of the inhibition zone in millimeters.

Test bacteria	Inhibition zone (mm) produced by AgNPs from	
	Raoultella spp.	C
Proteus mirabilis	R	R
Shigella soni	16	10
Salmonella typhi	19	R
Klebsiella pneumonia	24	R
Enterobacter spp	21	R
Serratiam arcescens	17	R
E. coli	23	R
Sphingomonas paucimobilis	15	10
Pseudomonas aeroginosa	31	R
Kocuria spp	11	R
Staphylococcus aureus	18	R

Table 7. The incubation period for the synthesis of silver nanoparticles (AgNPs) by Raoultella sp. Was optimized, with the resulting inhibition zone measured in millimeters.

Strain	Inhibition zones at					
	0h	4 h	10 h	24 h	28 h	34 h
Raoultella spp..	13	14	18	14	0	14

elevation indicates that the nanoparticles induced a prooxidant state rather than scavenging radicals, yielding a negative antioxidant index, the specific values of which are cataloged in (Table 8).

Antibiofilm activity

The propensity of pathogenic microorganisms to assemble robust biofilms on surgical instrumentation and indwelling prosthetics poses a severe clinical challenge, thereby driving significant interest in nanobiotechnological countermeasures To assess their preventative potential, a standard microtiter plate assay was deployed to compare the biofilm-disrupting capabilities of the Raoultella-derived AgNPs against standard commercial silver nanoparticles Testing was conducted across a tripartite dosage gradient targeting a diverse panel of five Gram-positive and Gram-negative clinical isolates The overarching data indicates that eradication efficacy is highly contingent upon both the target microbial species and the synthetic origin of the nanomaterial Species-specific analyses revealed complex, and occasionally non-linear, inhibition dynamics When applied to Shigella sonnei, both the biological and commercial formulations successfully degraded the biofilm matrix; however, this inhibitory action intriguingly displayed an inverse correlation with nanoparticle concentration A comparable inverse dose-response was noted for commercial AgNPs tested against Salmonella typhi, whereas the biogenic variant required the maximum dosage to achieve notable disruption In the case of Klebsiella pneumoniae, commercial particles exhibited optimal clearance at 3.0 mg/mL Conversely, the Raoultella-synthesized AgNPs demonstrated a biphasic efficacy profile, generating significant biofilm suppression at both the highest and lowest testing thresholds Treatment of Aeromonas sobria further highlighted these differing kinetic

Table 8. Antioxidant activity of the biogenic AgNPs fabricated by *Raoultella spp*.

Concentration of AgNPs(mg\ml)	Antioxidant Index (AI%)
3	-6.35362
1.98	-5.1498
1.5	-5.69806
0.75	-2.01567
0.00	0

Table 9. The antibiofilm potential of both commercial and bacteriogenically fabricated silver nanoparticles was assessed across a range of concentrations This evaluation, quantified by absorbance at 630nm, targeted their activity against various pathogenic Gram-positive and Gram-negative bacterial strains.

Bacteria	Concentration of AgNPs (mg/ml)	Cont.	Absorbance (630nm) after treatment with AgNPs	
			Commer.	*Raoultella spp.*
Shigella sonnei	3.00	0.883	0.470	0.301
	1.50		0.332	0.252
	0.75		0.280	0.249
Salmonella typhi	3.00	1.59	0.745	0.317
	1.50		0.536	0.463
	0.75		0.344	0.456
Raoultella spp..	3.00	2.784	0.221	0.511
	1.50		2.686	0.790
	0.75		2.692	0.458
Aeromonassobria	3.00	2.776	0.361	0.289
	1.50		0.248	2.751
	0.75		0.425	0.210
Staph. aureus	3.00	2.660	0.849	1.193
	1.50		1.285	2.104
	0.75		2.324	1.120

behaviors: commercial AgNPs reached peak performance at an intermediate dose of 1.5 mg/mL, while the biogenic particles were most suppressive at the lower 0.75 mg/mL boundary Finally, Staphylococcus aureus biofilms exhibited a direct, dose-dependent susceptibility to commercial AgNPs, yet the biological counterparts once again peaked in efficacy at 0.75 mg/mL Despite these varying kinetic responses, an aggregated review of the assay results strongly suggests that the biogenic AgNPs generally outperform their commercial equivalents in mitigating biofilm architectures (Table 9).

Morphological and elemental characterization of biogenic AgNPs

Physical characteristics of the silver nanoparticles was mapped comprehensively by scanning electron microscopy, energy-dispersive X-ray spectroscopy, atomic force microscopy, and X-ray diffraction to get the job done.

Scanning Electron Microscopy (SEM) analysis

Our comparative microstructural analysis

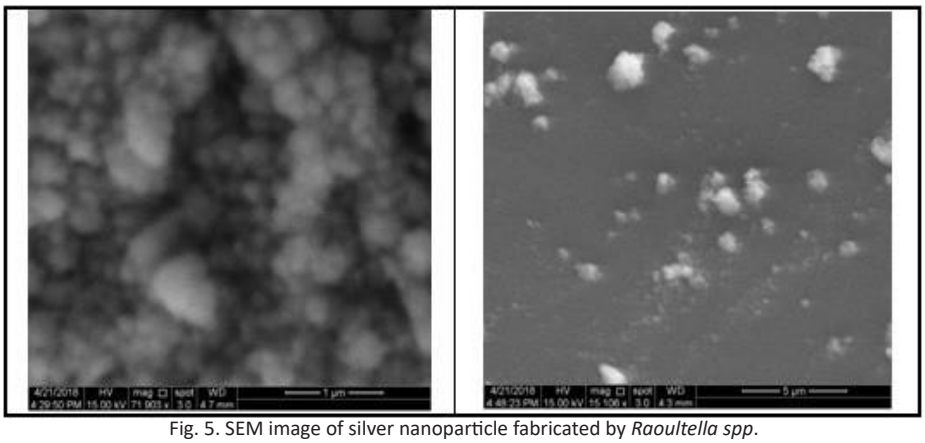

Fig. 5. SEM image of silver nanoparticle fabricated by *Raoultella spp*.

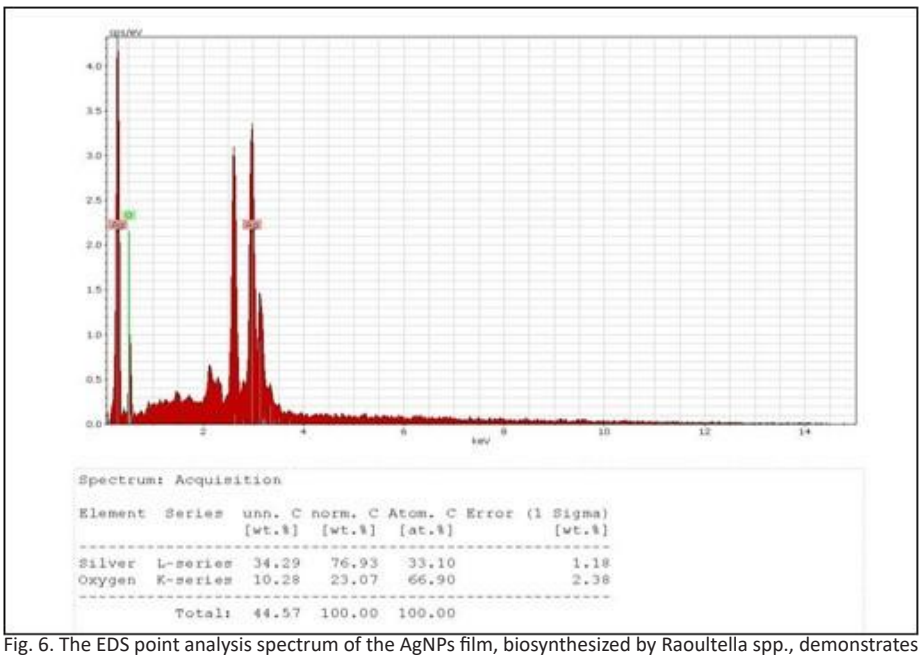

Fig. 6. The EDS point analysis spectrum of the AgNPs film, biosynthesized by Raoultella spp., demonstrates the presence and quantity of silver nanoparticles.

revealed some very distinct morphological traits when looking at nanoparticles synthesized across different bacterial strains. High-resolution SEM images of the AgNPs produced specifically by the *Raoultella spp.* isolate demonstrated a highly homogeneous and entirely monodisperse colloidal population The individual particles showed negligible aggregation, uniform spatial distribution, and were predominantly spherical in their geometry (Fig. 5).

Energy Dispersive X-Ray Spectroscopy (EDS)

Stoichiometric distribution and quantitative elemental composition determined by using spot-profile EDS spectral acquisition. The spectra obtained confirmed that there was successfully enzymatically mediated process to reduce the silver ions directly into zero-valent metallic silver If you look at Fig. 6, the spectral mapping exhibits a massive, dominant optical absorption peak centered perfectly at 3 keV This specific peak is the characteristic spectral fingerprint that corresponds to the surface plasmon resonance of metallic silver nanocrystals. When ran peak intensity analysis, it showed that silver was the primary constituent. This was accompanied by a moderate signal indicating atomic oxygen, alongside some very minor background traces of other biological elements. Stoichiometric quantification was ran on the Raoultella-derived nanoparticles This established that their elemental composition was exactly 76.93% silver and 23.07% oxygen by weight (Fig. 6).

Atomic Force Microscopy (AFM)

atomic force microscopy was employed to map out the roughness, average diameter, and overall morphology of the silver nanoparticles synthesized using Raoultella spp. ultimate size and shape of the resulting structures was controlled by carefully manipulating both the current density and the etching time. The AFM analysis ran on the biogenic AgNPs produced by Raoultella spp Indicated that they had an average diameter of exactly 62.88 nm (Fig. 7).

X-Ray Diffraction analysis (XRD)

The X-ray diffraction pattern of the biogenic silver nanoparticles (AgNPs) exhibited distinct peaks at 2θ values of 37.749, 43.916, 63.833, and 76.666, corresponding to the crystallographic planes indexed as 111, 200, 220, and 311, respectively (Fig. 8). These lattice planes were indexed consistently with the face-centered

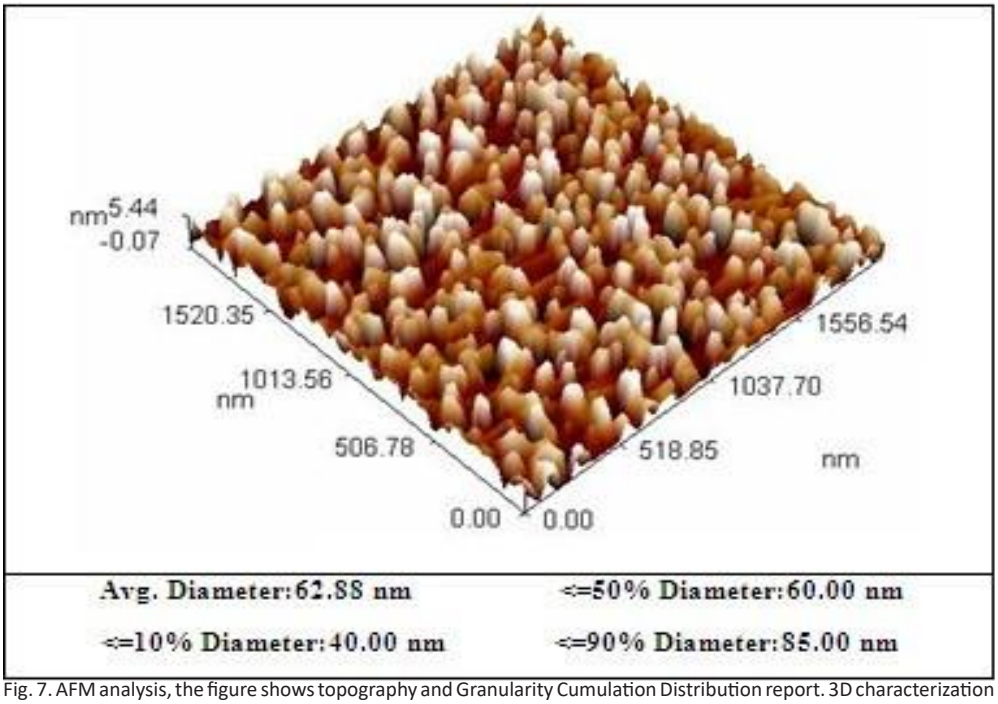

Fig. 7. AFM analysis, the figure shows topography and Granularity Cumulation Distribution report. 3D characterization of biogenic AgNPs fabricated by *Raoultella spp*.

cubic (FCC) silver structure, as corroborated by JCPDS data file number 04-0783. Employing the Debye-Scherrer equation, the average crystallite size of the AgNPs fabricated by Raoultella spp. Was determined to be 21.918 nm. The XRD diffractograms indicate that Raoultella spp. Produces AgNPs with an average crystallite size of 21.9 nm, an average dislocation of 39.33, and an average of 21.22.

Screening of bacteria for AgNPs biosynthesis

Habitats that are heavily contaminated with toxic heavy metals put an immense amount of selective pressure on the local environment This naturally favors microflora that are already equipped with specialized genetic and biochemical detoxification pathways These adaptive mechanisms are incredibly critical for ecological bioremediation, but they also offer a highly unique platform for nanobiotechnology Researchers can actually harness the way these microorganisms mitigate metal-induced oxidative stress can deploy them as living biological factories to handle the controlled synthesis of metallic nanomaterials During this study, nanobiosynthesis pipeline was successfully optimized by systematically screening various soil isolates until ultimately identified *Raoultella spp.* as an exceptional candidate. The well-documented metabolic plasticity of this genus really reinforces its suitability for this kind of work. It is particularly known for its robust nitrogen fixation and assimilation networks, which are driven by very distinct genetic elements like the nif and nacK gene clusters [23, 24].

Optimization of AgNPs biosynthesis

The kinetic efficiency of generating nanoparticles is intrinsically tied to the specific catalytic environments that microbial reducing enzymes demand Over the course of this study experiments, its demonstrated that *Raoultella spp.* hit its maximum AgNP yield when exposed to a 1 mM $AgNO_3$ precursor at 37°C when kept it in oppace and only ran a brief 10 hours incubation. The classic chromogenic shift of the broth provided macroscopic validation of this bioreduction. Its watched transcoloration from a pale yellow color directly into a deep reddish-brown. Researchers currently favor extracellular synthesis protocols over intracellular extraction. This is mostly because it drastically simplifies the downstream purification of the final product Inside our system, the cell-free supernatant pulls double duty It acts dualistically as the stabilizing capping agent and the reducing matrix. The enzymes were postulated to be like nitrate reductase work synergistically with the reactive aldehyde groups found on secreted polysaccharides to drive the reduction

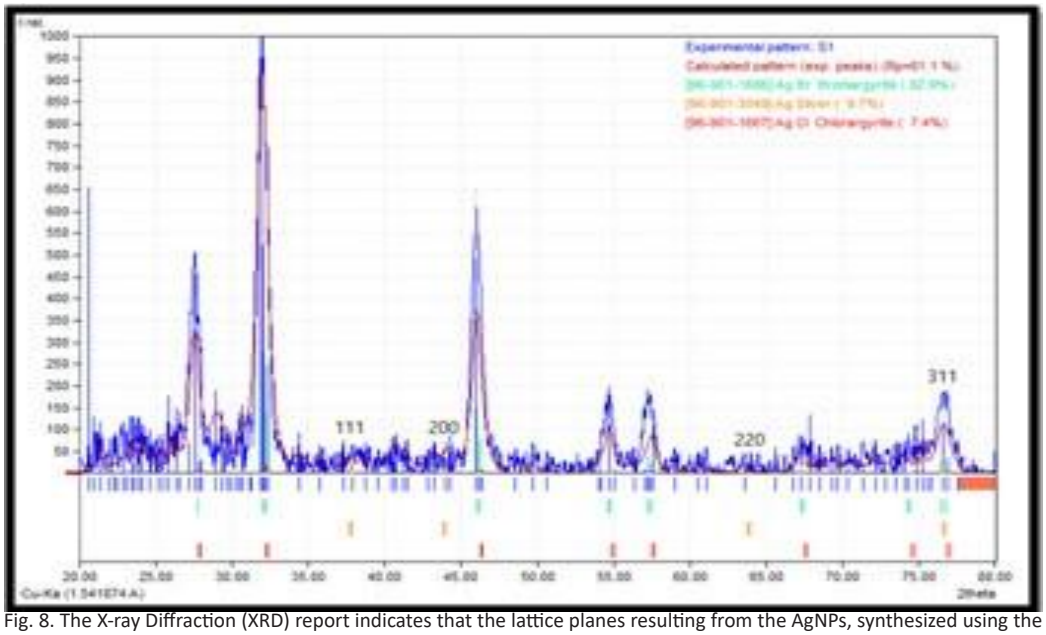

Fig. 8. The X-ray Diffraction (XRD) report indicates that the lattice planes resulting from the AgNPs, synthesized using the *Raoultella spp* strain, correspond to JCPDS data file number (04-0783).

of Ag+ to Ag0. A remarkably rapid 10 hours production window was observed during this study This aligns perfectly with recent literature and might actually be functionally linked to the very robust sil gene cluster that governs silver resistance in this specific isolate It is important to remember that the catalytic efficiency and tertiary structure of these biomolecules are extremely sensitive to environmental fluctuations Because of this, you absolutely have to tightly regulate both pH and thermal conditions Interestingly, particle nucleation showed a highly non-linear response to pH changes Strongly acidic conditions severely handicapped the reductive process, while highly alkaline environments completely maximized nanoparticle proliferation [25].

Biological characterization of biogenic AgNPS
Antimicrobial activity

The global crisis surrounding multidrug-resistant pathogens is rapidly escalating This has severely catalyzed the search for brand new, non-traditional therapeutic agents Silver nanoparticles have stepped up as a formidable alternative They are fully capable of functioning completely independently, or they can work synergistically alongside traditional antibiotics to tear down microbial defenses By utilizing agar well diffusion assays, it was noted that the Raoultella-derived AgNPs exhibited highly variable, yet undeniably potent, bactericidal effects This held true across a wide spectrum of both Gram-negative and Gram-positive targets, with the sole exception being Proteus mirabilis These noticeable variations in inhibition really underscore a critical point Antibacterial efficacy is heavily dictated by the specific biological origin of the capping agents used. On top of that, the differential susceptibility seen between the Gram-negative and Gram-positive cohorts comes down to basic biology It is largely governed by species-specific quorum sensing networks and fundamental disparities in their cell envelope architectures Even if you look within closely related bacterial clades, their overall tolerance fluctuates This happens based on the functional efficiency of their membrane efflux pumps, their inherent genomic resistomes, and whether or not they carry specialized resistance plasmids Ultimately, it is the morphological profile of the nanoparticles that dictates how they interact with bacterial targets This includes their specific molecular corona, dispersity, geometry, and size [26, 27].

Antioxidant activity

Radical scavenging competency of biogenic AgNPs was assessed, by standard 2,2-diphenyl-1-picrylhydrazyl colorimetric assay. Mechanistically speaking, this test relies on the physical reduction of the DPPH radical Because it holds an unpaired electron, DPPH exhibits a very strong intrinsic absorbance peak right at 517 nm When it finally accepts an electron from an antioxidant, the entire solution typically shifts away from a deep violet color and turns into a pale yellowish-orange This causes an absorbance drop that directly and inversely correlates with the overall antioxidant strength of the material Completely unexpectedly, our spectrophotometric readings showed absorbance values that either matched or outright exceeded the baseline control This anomaly likely doesn't mean the antioxidant capability is missing entirely Instead, it is almost certainly a byproduct of the biochemical composition of the Raoultella supernatant itself, which frequently carries pigmented carotenoids and specific exopolysaccharides [16] elucidated exactly why this happens, noting that the DPPH methodology suffers from two massive limitations in nanobiological settings First, the nanoparticles are encased by bulky biomolecular capping proteins These proteins cause substantial steric hindrance, which physically blocks access to the centrally located nitrogen radical sitting on the DPPH molecule Second, biological pigments like carotenoids naturally possess a massive amount of light absorption right near that critical 517 nm wavelength. This spectral overlap artificially inflates all the absorbance data, which effectively masks any genuine free radical scavenging events that might be happening [17].

Antibiofilm Activity

Pathogenic biofilms are basically dense microbial communities encased inside a viscous, highly protective extracellular matrix. They pose a severe clinical challenge because they enable bacteria to adhere to medical prosthetics and host tissues while simultaneously skyrocketing their antimicrobial resistance. Traditional antibiotics simply struggle to penetrate this polymeric shield Because of this, researchers are rigorously investigating AgNPs as potential matrix-disrupting agents, The biogenic AgNPs was compared their

biofilm-eradicating capabilities directly against commercial variants. They were tested across three different concentration gradients, targeting five totally distinct clinical isolates using a microtiter plate assay. Analysis of the experimental data revealed significant heterogeneity in the efficacy of biofilm suppression. Success was entirely contingent upon the synthetic source of the nanoparticles and the identity of the target pathogen Contemporary models actually suggest that AgNPs dismantle biofilms by triggering a multifactorial cascade. They suppress extracellular polymeric substance secretion, physically alter surface adhesivity, and directly downregulate the complex genetic networks responsible for quorum sensing communication [25, 26].

Characterization of Biogenic AgNPs

Both the compositional and structural identities of the Raoultella-synthesized AgNPs was confirmed by combining basic macroscopic observation with highly advanced instrumental analytics. This included XRD, AFM, EDS, and SEM The chromogenic shift of the medium provided our initial validation of extracellular synthesis This shift acts as a direct, undeniable optical manifestation of surface plasmon resonance activation happening among the newly formed nanoparticles Following that, microstructural mapping was performed using Scanning Electron Microscopy. This illustrated a highly dispersed population composed of spherical nanostructures. This kind of geometric uniformity is heavily dictated by the exact biomolecular constituents floating in the reaction broth. Those constituents are what actually modulate the particle nucleation and growth dynamics. The elemental validation was achieved by utilizing Energy-Dispersive X-ray Spectroscopy. A rigorous stoichiometric analysis of the generated particles confirmed their exact composition: 23.07% oxygen and 76.93% silver by weight. The raw intensity of the silver signal aligns perfectly with the anticipated SPR signature of metallic nanocrystals. This definitively confirms the successful bio-reduction of ionic silver straight into its elemental state. At the same time, that robust oxygen signal strongly implies that these nanoparticles are being sterically stabilized by oxygen-rich biological macromolecules passed down from the culture medium. The minor background peaks were attributed to lingering residual organic cellular metabolites. Finally, we interrogated the crystallographic architecture using X-ray Diffraction. The resulting diffractogram revealed four very distinct Bragg reflections positioned flawlessly at the diffraction peaks corresponding to the (111), (200), (220), and (311) planes. This highly specific diffraction profile is the definitive, undeniable signature of a face-centered cubic crystal lattice, which is completely characteristic of pure elemental silver. By applying the Debye-Scherrer equation directly to these reflection data, we were able to calculate the mean crystallite diameter of Raoultella-synthesized AgNPs to be exactly 21.918 nm [22].

CONCLUSION

This study successfully demonstrated the eco-friendly, extracellular biosynthesis of silver nanoparticles (AgNPs) utilizing a heavy-metal-resistant *Raoultella* soil isolate, molecularly confirmed via *rpoB* gene sequencing. Operational parameters were optimized to maximize yield, establishing ideal reaction conditions at 37 °C, alkaline pH, and a 10-hour incubation window under dark conditions. Comprehensive physicochemical characterization confirmed the formation of stable, monodisperse, spherical nanostructures. Electron microscopy (SEM/AFM) revealed an average hydrodynamic diameter of 62.88 nm, while X-ray diffraction (XRD) confirmed a face-centered cubic metallic lattice with a mean crystallite size of 21.9 nm. Energy-dispersive X-ray spectroscopy (EDS) verified the elemental zero-valent silver core (76.93% Ag) stabilized by biological capping moieties. Functionally, the biogenic AgNPs displayed exceptional antibacterial potency against multidrug-resistant pathogens—yielding pronounced inhibition zones against *Pseudomonas aeruginosa* (31 mm) and *Staphylococcus aureus* (18 mm)—surpassing commercial counterparts. Furthermore, microtiter assays underscored potent species-specific antibiofilm disruption at low dosages. Although steric capping and residual biopigments confounded standard DPPH scavenging metrics, the pronounced biocidal and anti-adherence performances highlight these bio-fabricated AgNPs as sustainable, potent therapeutic candidates to counteract microbial biofilm formation and antibiotic resistance.

CONFLICT OF INTEREST

The authors declare that there is no conflict

of interests regarding the publication of this manuscript.

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