# Bacteriogenic Synthesis of Morphologically Diverse Silver Nanoparticles and their Assessment for Methyl Orange dye removal and antimicrobial activity

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**Abstract**

In the last decade nanotechnology and nanoparticles have attracted the attention of the scientific community in the whole world. The microbial approaches for the synthesis of nanoparticles are more economical, biocompatible, and environment-friendly than the chemical and physical approaches. In the present research work, investigators have synthesized three different types of silver nanoparticles (AgNPs), namely AgNPs-K, AgNPs-M, and AgNPs-E, by using *Klebsiella pneumoniae, Micrococcus luteus,* and *Enterobacter aerogenes,* respectively. The morphological, chemical, and elemental features of the synthesized AgNPs were analyzed by using UV-Vis spectroscopy (UV-Vis), Fourier transform-infrared spectroscopy (FTIR), X-ray diffraction (XRD), field emission scanning electron microscope (FESEM) and energy-dispersive spectroscopy (EDX). UV-Vis absorbance peaks were obtained at 475 nm, 428 nm, and 503 nm for AgNPs synthesized by, *K. pneumoniae, M. luteus,* and *E. aerogenes* respectively. The XRD showed the crystalline nature of the synthesized AgNPs, having peaks at 26.2°, 32.1°, and 47.2°, while the FTIR showed bands at 599 $cm^{-1}$, 963 $cm^{-1}$, 1693 $cm^{-1}$, 2299 $cm^{-1}$, 2891 $cm^{-1}$, and at 3780 $cm^{-1}$ for all the types of AgNPs. The FTIR indicated the presence of attached biomolecules from bacteria with developed AgNPs. The size of the AgNPs varies from 10 nm to several microns while the shape varies from spherical to porous sheets-like structure. The percentage of Ag varied from 37.8% (wt.%) to 61.6% i.e., highest in AgNPs-K, and lowest in AgNPs-M. Further, all three types of AgNPs were evaluated for the removal of methyl orange dyes from the simulated wastewater where the highest dye removal percentage was 19.24% at 120 minutes by AgNPs-M. Finally, all three types of AgNPs were assessed for their potential for antibacterial activity against Gram-positive bacteria (*Bacillus subtilis, B. cereus*, and *B. megaterium*) and Gram-negative bacteria (*Enterococcus fecalis*), out of which the largest zone of inhibition was 12 mm against *B. megaterium* for the AgNPs-M.

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**KEYWORDS** silver nanoparticle, methyl orange, bioremediation, antimicrobial, zone of inhibition

## 1. Introduction

The rapid industrialization in India as well as in the whole world has increased the use of different synthetic dyes (Wang et al., 2023a). Some of the dyes may cause harm to aquatic life and cause diseases in living organisms (Patel et al., 2022). Dyes are mainly used in textile industries for coloring fabric, so textile industrial wastewater acts as a major source of dye effluent (Al-Tohamy et al., 2022). The prolonged and continuous mixing of dye-laden water and dye effluents in the freshwater may lead to water pollution (Patel et al., 2022; Agarwal et al., 2022). The consumption of dye-contaminated water may cause numerous diseases in humans, like skin irritation and skin cancer, in the long term. Dyes present in textile effluent can be removed by using various chemical approaches like precipitation, coagulation (Yadav et al., 2022b; Wang et al., 2023c), flocculation, membrane filtration (nanofiltration, ultrafiltration) (Wang et al., 2020; Chahar et al., 2023; Zhang et al., 2023), reverse osmosis, adsorption, etc. (Robati et al., 2016). The biological methods involve the utilization of microorganisms (Gupta et al., 2022) for dye remediation, either in the natural sites or in the bioreactor in the laboratory (Das & Mishra, 2017; Singh et al., 2023). The biological approach also involves biosorbents for the remediation of dyes from wastewater (Cui et al., 2017; Modi et al., 2023). Such processes are economical if the biosorbents are developed from agricultural waste, etc. All of these processes have certain advantages and disadvantages, but adsorption is a very simple, effective, and economical approach. The adsorbent in the adsorption process could be easily surface functionalized by various chemical compounds for the targeted removal of pollutants (Cui et al., 2013; Chen et al., 2020; Harja, Buema & Bucur, 2022). The various adsorbents that are commonly used for the remediation of dyes and other pollutants from contaminated water are alumina, silica (Imoisili, Nwanna & Jen, 2022; Yadav et al., 2023),

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zeolites (Murukutti & Jena, 2022), coal fly ash, magnetite, maghemite, zinc oxide (Soltani et al., 2023), titanium dioxide (Dash et al., 2018; Yang, Shojaei & Shojaei, 2022), and other complexes (Cui et al., 2017). When these adsorbents are used in their nanoform, they become highly effective due to their high surface area to volume ratio (SVR) and high surface energies (Chen et al., 2020).

Nanotechnology has played a significant role in environmental clean-up, especially the removal of textile dyes from contaminated water. Among NPs, metallic, metal oxide NPs, and nanocomposites Among pure metallic NPs, silver nanoparticles (AgNPs) have gained huge popularity as they are effective in killing waterborne pathogens due to their antimicrobial properties (Choudhary, Pathak & Madhusudan, 2017). The size of the NPs mainly falls in the range of 1 to 100 nm (Puri, Gupta & Mishra, 2021), which has gained huge popularity in the field of adsorption-based removal of pollutants from wastewater due to their SVR. Moreover, NPs have a high adsorption capacity, due to which dyes easily get adsorbed on the surface of NPs (Degefa et al., 2021; Tarekegn et al., 2021; Wang et al., 2023a).

AgNPs could be synthesized by all three possible methods, i.e., chemical, physical, and biological (Bouafia et al., 2021). The chemical approaches include chemical coprecipitation (Adibah, Firdianti & Suprapto, 2023), sol-gel (Shahjahan et al., 2017), chemical reduction (Horne et al., 2023), polyol (Wolf et al., 2022), etc. Among physical approaches, the most familiar ones are ball milling (Lai et al., 2023), the vapor condensation method (Simchi et al., 2007), arc discharge (El-Khatib et al., 2018), and laser-ablation techniques (Rafique et al., 2019; Juma et al., 2023). The chemical approaches involve the utilization of hazardous chemicals, which are not environmentally friendly, while the physical ones utilize of expensive instruments, which makes the synthesis highly expensive. The biological synthesis techniques, i.e., phytonanofabrication (Choudhary et al., 2023a) and microbial synthesis of AgNPs, are of high significance due to the lower utilization of chemicals and the absence of the requirement of

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a chemical capping agent or surfactant. The microbial synthesis methods provide natural stabilizing and capping agents, which make them biocompatible (Naganthran et al., 2022). Among all the microorganisms, bacterial synthesis is preferred due to their easy handling and short time duration for their growth in comparison to algae and fungi (Wang et al., 2023b; Choudhary et al., 2023b). Bacteria are enriched with several bacterial enzymes and proteins, which play an important role in the bio-reduction of $Ag^{2+}$ ions into $Ag^{0}$. These biological molecules act as reducing agents, capping agents, and stabilizing agents for the developed AgNPs. Vimalanathan and his team synthesised silver nanoparticles (AgNPs) using the moist biomass of *Micrococcus luteus* (Vimalanathan et al., 2013). To date, several investigators have used potential bacteria for the formation of AgNPs for instance, Esmail and his colleagues synthesized AgNPs (25 nm), by using the supernatant of the bacteria *Bacillus* ROM6. This particular bacterium was isolated from the Zarshouran gold mine in South Korea. Further, the synthesized AgNPs were used as an antimicrobial agent against *Escherichia coli*, *Acinetobacter baumannii, Staphylococcus aureus,* and *Pseudomonas aeruginosa* (Esmail et al., 2022).

From the very beginning of civilization, the antimicrobial effect of Ag was known due to which it was used for various applications (Kyung et al., 2008). Being a heavy metal, it coagulates the enzymes and proteins of the microorganism, thus inhibiting them and ultimately killing them (Betts, Whitehead & Harris, 2021). So, the nanosized silver can increase the efficiency of antimicrobial activity due to its small size, and high SVR, as it is smaller in size, it may enter the microorganism through the cell wall, resulting in the inhibition and killing of the microorganism (Karunakaran et al., 2017). Several research studies have revealed that the AgNPs possess incredible antimicrobial activity (Singh & Mijakovic, 2022) which depends upon their size and surface area (Kalwar & Shan, 2018). The smaller size of AgNPs may facilitate their entry into the microbes and exhibit their antimicrobial effect. There are several examples where AgNPs have been used as an antimicrobial agent.

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Saeed and co-researchers synthesized spherical-shaped AgNPs of size 5-50 nm using bacterial strains; *E. coli*, *Exiguobacterium aurantiacumm*, and *Brevundimonas diminuta*. The investigators further, observed the antimicrobial activity of the synthesized AgNPs against methicillin-resistant *Staphylococcus aureus* (MRSA) and several other multiple drug resistance (MDR) bacteria, where the zone of inhibition (ZOI) varied from 10 mm to 28 mm. Further, the investigators utilized AgNPs against plant pathogens (Saeed, Iqbal & Ashraf, 2020). A research group led by Cekuolyte synthesized morphologically different types of AgNPs by using different strains of *Geobacillus* bacteria, namely 18, 25, 95, and 612 (Cekuolyte et al., 2023). A research group led by Raza synthesized AgNPs by using *Aspergillus fumigatus* KIBGE-IB33 and evaluated the antimicrobial activity of AgNPs on *Enterococcus faecalis* ATCC 29212. Further, the investigators developed a nanocomposite by using the AgNPs with chitosan and observed that the lowest minimum inhibitory concentration of the nanocomposite system was 1.56 µg $mL^{-1}$ against *Enterococcus faecalis* ATCC 29212 (Raza et al., 2021). Srinivasan and their colleagues synthesised silver nanoparticles (AgNPs) using a bioluminescent bacterium (*Vibrio campbellii*).

Further, the investigators assessed the antibacterial and antioxidant properties of the synthesized AgNPs. The antibacterial potential was evaluated against several pathogenic Gram-negative bacteria (GNB) like *Aeromonas hydrophila* MTCC 1739, *Klebsiella oxytoca* MTCC 3030, *K. pneumoniae* MTCC4030, and *Ps. aeruginosa* MTCC 1934. The AgNPs exhibited antioxidant features through strong scavenging actions on 2,2-diphenyl-1-picrylhydrazyl (DPPH) (61.88%) and $H_2O_2$ (53.48%) free radicals (Srinivasan et al., 2022). From the above investigation, it was found that there was no information on the morphology of AgNPs synthesized by different bacteria. Moreover, AgNPs were used in nanocomposite form, so individual potential assessments of AgNPs. So, a study is needed to investigate the effect of different bacteria on the morphology of AgNPs and their

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purity. Moreover, the antimicrobial effect of morphologically diverse AgNPs on the pathogenic bacteria.

From all the above investigations, various gaps were found, which are mentioned below: i) the effect of different bacterial strains and their enzymes on the morphology of the synthesis of AgNPs; ii) the utilization of AgNPs along with composite material to form nanocomposite for antimicrobial properties could not reveal the effectiveness of the antimicrobial properties of AgNPs in the composite. So, to understand these two above-mentioned issues, a detailed investigation is needed to study the effect on the morphology of AgNPs and their purity by different bacteria and their enzymes, as well as the antimicrobial potential effect of morphologically diverse AgNPs on the pathogenic GPB and GNB.

Gola and their group synthesized 6-25 nm, spherical, and hexagonal-shaped AgNPs from *Aspergillus sps.* Further, the synthesized AgNPs were used for the removal of reactive yellow dye and antibacterial potential (Gola et al., 2021). A research group led by Rasheed synthesized AgNPs from *Conocarpus erectus* and *Pseudomonas* sp. and applied them to the elimination of reactive black 5 (RR5) and reactive red 120 (RR120) from the aqueous solutions (Rasheed et al., 2023).

Here, the investigators have harnessed the potential of Gram-positive bacteria (*Micrococcus luteus*) and Gram-negative bacteria (*Klebsiella pneumoniae* and *Enterobacter aerogenes*) for the synthesis of silver nanoparticles (AgNPs) under normal laboratory conditions. One of the objectives was to confirm the formation of AgNPs, along with purity and morphology, by using Fourier transform-infrared (FTR-IR), UV-Vis spectrophotometer (UV-Vis), X-ray diffraction pattern (XRD), Field emission scanning electron microscopy (FESEM), and Energy dispersive X-ray spectroscopy (EDS). AgNPs. Another objective was to observe the morphological and elemental diversity among the bacterially synthesized AgNPs. Another objective was to

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evaluate the potential of AgNPs as an adsorbent for the removal of methyl orange dye from aqueous solutions. The final objective was to evaluate the potential of the synthesized AgNPs as an antibacterial agent against GPB (*B. subtilis, B. cereus, and B. megaterium*) and GNB (*Enterococcus fecalis*).

## 2. Materials and methods

### 2.1. Materials

*K. pneumoniae, M. luteus, E. aerogenes, B. subtilis, B. cereus, B. megaterium,* and *Enterococcus fecalis* were procured from the Gujarat Biotech Research Centre, Gujarat, India, silver nitrate (SRL, Gujarat, India), nutrient agar media (Himedia, Mumbai, India); nutrient broth (Himedia, Gujarat, India); antibiotic assay media (Himedia, Mumbai, India); ethanol (Shenzhen, China), Whatman filter paper no. 42. (Axiva, Mumbai, India); and methyl orange (Loba, Chemie, Gujarat, India). All the chemicals were of analytical grade except silver nitrate and methyl orange dye (LR grade), and double distilled water (ddw).

### 2.2. Methods

#### 2.2.1. Screening and selection of bacteria for the synthesis of AgNPs

Around 10 bacterial colonies were procured on nutrient agar Petri plates, which were stored in a refrigerator in the laboratory. Further, about 200 mL of nutrient broth was prepared, to which about 1 mM of an aqueous solution of $AgNO_3$ was added. Further, about 10 mL of this mixture was transferred into 10 different Erlenmeyer flasks of 50 mL. To all these flasks, a loopful culture of each bacterial strain was added and incubated in an incubator shaker at 37 °C for 2-3 days. After incubation, a color change was noticed, and later on, UV-Vis spectra were taken for all the samples. Out of all these bacterial strains, only three were found positive for the AgNPs synthesis as the color change to red and an absorbance peak near 500-540 nm was observed. So further, only these three bacterial strains were used for the large amount of AgNPs

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formation. These bacterial strains were identified by 16S rRNA genome sequencing. The selected bacterial strains were *M. luteus, K. pneumoniae,* and *E. aerogenes.*

**2.2.2. Synthesis of silver nanoparticles from bacteria**

For the fabrication of AgNPs, silver salt was reduced by the bacterial supernatants obtained from all three bacterial strains. The isolated bacterial colonies were grown on nutrient agar plates. Further, for the mass production of each bacterial colony, a loopful of culture was inoculated into the nutrient broth in three separate Erlenmeyer flasks. All three flasks containing nutrient broth were incubated in an incubator shaker at 37 ℃ for 24 hours at 120 rpm. Further, after 24 hours, the bacterial colonies were taken out and centrifuged at 5000 rpm for 10 minutes. The bacterial supernatant was retained, while the bacterial pellet was discarded. Further, about 100 mL of all three bacterial supernatants were taken separately in three amber bottles, and to each bottle, about 100 mL of silver nitrate solution was added. After that, all three flasks, including the control, which has ddw instead of bacterial supernatant, were kept under dark conditions for 2-3 days, and color change was continuously monitored. Initially, the color of the silver nitrate aqueous solution was pale, but after the addition of bacterial supernatant, the color tuned to a milky white in appearance. Finally, after 2-3 days, the color of the three bottles changed from milky white to reddish brown, indicating the formation of AgNPs. The mixture from each bottle was transferred to the centrifugation tubes separately and centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded, while the solid particle was retained. Further, the pellet was washed 2-3 times with distilled water and once with ethanol. All three types of AgNPs were then transferred to different Petri plates and kept for drying in an oven at 50-60 ℃ untill complete dryness. **Figure 1** shows the schematic steps

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**FIGURE 1** Schematic diagram for the synthesis of AgNPs using bacterial supernatant. The first step involves the growth of bacterial culture. The second step involves the lysis of the bacterial cells by centrifugation. The third step involves the addition of silver ion precursors to all the bacterial supernatant. The fourth step involves color change observation and confirmation by UV-Vis. The final step involves the recovery, washing, and drying of the AgNPs.

involved in the development of AgNPs from the bacterial supernatant.

### 2.2.3. Preparation of aqueous solution methyl orange dye

A 50-ppm aqueous solution of methyl orange (MO) dye was prepared by adding 50 mg of MO dye powder granules into the 1000 mL ddw. The aqueous solution was kept on a magnetic stirrer with vigorous stirring at 250 rpm to completely dissolve the dye granules. Further, Whatman filter paper was used for the filtration of the aqueous solution to eliminate the impurities. Finally, the dye sample was placed in an amber-colored glass reagent bottle for future use.

### 2.2.4. Batch study of adsorption of methyl orange dye

About 100 mL of an aqueous solution of MO dye was taken from the stock solution into three different glass beakers of appropriate volume. All three glass beakers were placed on a magnetic stirrer, and 1 mg of AgNPs of each type was added to different glass beakers. The interaction between the AgNPs and MO dye was carried out by agitation at 400 rpm for all three flasks. Further, an aliquot (2-3 mL) was collected from all three glass beakers at 0 minutes, 30, 60, 90, and 120 minutes. All the collected samples were then analyzed by the UV-Vis spectrophotometer to identify the concentration of the dye samples. The UV-Vis absorbance maxima of MO dye are 520±15 nm. Further, MO dye removal percentage was

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measured by using the following formula as provided by (Swathilakshmi et al., 2022) in Equation (1):

$$\% \, Dye \, removal = \frac{Co - Ct}{Co} \times 100 \qquad (1)$$

Where, $C_o$= initial dye concentration,

$C_t$= dye concentration at a specific time

**2.2.5. Antimicrobial activity of silver nanoparticles**

The antimicrobial properties of all the bacterially developed AgNPs (AgNPs-K, AgNPs-M, and AgNPs-E) were assessed against GPB: *B. subtilis, B. cereus, B. megaterium,* and GNB: *E. faecalis* by the disc diffusion method (Yassin et al., 2022). Firstly, 16 discs of specific size (8 mm diameter) were cut out of filter paper and dipped into separate reagent vials containing AgNPs-K, AgNPs-M, and AgNPs-E. Further, all the reagent vials were sonicated for 15-20 minutes using an ultrasonicator (Lequitron). Further, the AgNPs loaded discs were taken out of the vials with the help of forceps kept on three different Petri plates and dried in a hot air oven at 40-50 ℃. Further, autoclaved antibiotic assay media was prepared, in which all four tested bacteria were spread with the help of a sterilized cotton swab on different plates under aseptic conditions. The dried AgNPs loaded discs (three disks in one plate) were gently placed on the bacteria-swabbed Petri plates. Finally, the Petri plates were incubated overnight in a bacterial incubator at 37 ℃. After 24 hours, antibiotic assay plates were observed for the evaluation of the antimicrobial features of the formulated AgNPs. The ZOI was measured by using a measurement scale against the light, and the size was recorded in mm (Ballén et al., 2021).

**3. Characterization of silver nanoparticles**

**3.1. UV-Visible spectroscopy**

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The UV-Vis measurement of AgNPs was done by dispersing about 1 mg of all three types of AgNPs in 5 mL of ddw in three different test tubes. All three tubes containing AgNPs were sonicated in an ultrasonicator for 10 minutes to disperse the particles. The well-dispersed samples were then taken in a quartz cuvette, and the UV-Vis measurement was done in the range of 200-800 nm at a resolution of 1 nm by using a UV-Vis spectrophotometer (UV 1800, Shimadzu spectrophotometer, Japan).

### 3.2. FTIR

The FTIR measurement was done to identify the various functional groups present in the bacterially synthesized AgNPs. The FTIR measurement was done using the solid KBr pellet method, where the pellets were prepared by mixing 2 mg AgNPs and 198 mg KBr for all three types of AgNPs. The measurement was done in the mid-IR region 599-4000 $cm^{-1}$ at a resolution of 2 $cm^{-1}$ by using a spectrum S6500 instrument (Perkin-Elmer, USA).

### 3.3. XRD

The XRD patterns for all three types of AgNPs samples were recorded using a Miniflex 800 (Rigaku, Netherlands) instrument equipped with an X'celerometer to reveal to reveal the crystallinity. XRD patterns were recorded in the 2-theta range of 20-70 by using a filter K-beta (x1) with a step size of 0.02 and a time of 5 seconds per step, scan speed/duration time: 10.0 degree/min., step width: 0.0200 degree at 30 kV voltage, and a current of 2 mA.

### 3.4. FESEM-EDS

The morphological analysis of all three types of AgNPs was investigated using a Novo Nanosem, Fei 450, (USA). The dry AgNPs were loaded on the carbon tape with the help of a fine brush, which in turn was kept on the Al stub holder. All the samples were exposed to gold sputtering. The elemental analysis of AgNPs was carried out by using an Oxford-made energy-

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dispersive X-ray spectroscopy (EDS) analyzer fixed to the FESEM at variable magnifications at 20 kV.

## 4. Results and discussion

### 4.1. Mechanism of formation of AgNPs by bacteria

The bacterial strains, i.e., *K. pneumoniae*, *M. luteus*, and *E. aerogenes*, have numerous microbial proteins and enzymes that help in the bioreduction of $Ag^{2+}$ ions into $Ag^0$ (Ballén et al., 2021). The actual mechanism of the biosynthesis of AgNPs by bacteria is well described in the literature. It is a very simple and easy mechanism where the oxidized silver ions get two electrons from any of the microbial proteins and enzymes and get reduced to the stabilized $Ag^0$. When the $AgNO_3$ aqueous solutions are mixed with the bacterial culture/supernatant, the bacterial enzymes present in the supernatant reduce the $Ag^{2+}$ ions into $Ag^0$. So, during this step, the previously milky color of the aqueous silver solutions gets converted to a red color, which indicates the development of AgNPs in the medium. Moreover, these biomolecules may also act as stabilizing and capping agents for the synthesized AgNPs (Giri et al., 2022; Terzioğlu et al., 2022). **Figure 2** shows the mechanism involved in the formation of AgNPs from the bacteria. Here, the color of the medium changed from milky white to dark brown within 2-3 days. Earlier, a similar color change (yellow to brown) was observed during the synthesis of AgNPs by *K. pneumoniae* isolated from humans and sheep (Sayyid & Zghair, 2021). Saleh and Alwan (2020) used *K. pneumoniae* culture supernatant for the biosynthesis of AgNPs (Saleh & Khoman Alwan, 2020). Earlier, Javaid et al. also suggested a similar pathway for the formation of AgNPs from the bacteria via a NADH-dependent nitrate reductase enzyme (Javaid et al., 2018). **Table 1** shows the major microbial proteins and enzymes present in *K. pneumoniae, M. luteus,* and *E. aerogenes*.

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**TABLE 1 The major microbial proteins and enzymes present in *K. pneumoniae, M. luteus,* and *E. aerogenes*.**

**FIGURE 2** Schematic diagram for the development of AgNPs from silver ions by bacteria via the NADH-dependent nitrate reductase enzyme. The oxidized silver ions interact with the bacterial NADPH-dependent nitrate reductase enzyme, leading to the reduction of the $Ag^{2+}$ ions. The silver ions get capped with biomolecules from the bacteria. Further, these silver ions grow and nucleate, which get capped and stabilized by bacterial biomolecules, leading to the formation of stabilized AgNPs.

### 4.2. UV-Vis analysis for preliminary confirmation of the formation of AgNPs

**Figure 3** shows the typical UV-Vis spectra of AgNPs synthesized by bacteria. All of them exhibit a peak in the range of 425-505 nm. These absorbance peaks indicate the formation of AgNPs from the $Ag^{2+}$ ions by the bacteria (Saleh & Khoman Alwan, 2020). Earlier, Saleh and Alwan (2020) obtained a peak at 432 nm for the AgNPs synthesized by *K. pneumoniae* (Saleh & Khoman Alwan, 2020), 420 and 440 nm by *M. luteus* (Vimalanathan et al., 2013), 450 nm by cyanobacterium *Oscillatoria limnetica* (Hamouda et al., 2019), 450 nm by endophytic bacteria *Enterobacter roggenkampii* BLS02 (Kumar & Dubey, 2022), and 405-407 nm for *Klebsiella pneumoniae* by the research group of Kalpana. The investigators concluded that when the ratio of $AgNO_3$ to bacterial supernatant was about 4:6, the absorption intensity was higher, so they formed AgNPs. It was further concluded that the UV–Vis absorption is directly proportional to the amount of substance at their maximum absorption spectra, i.e., a higher yield of AgNPs and efficient production of AgNPs even at lower concentrations of silver nitrate can be obtained when more amount of cultural filtrate is used. The investigator further showed that the amount of reducing agents plays an important role in the formation of AgNPs (Kalpana & Lee, 2013).

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**FIGURE 3** UV-Vis measurement of AgNPs synthesized by bacteria.

### 4.3. Identification of functional groups of silver NPs by FTIR

A typical FTIR spectra of all the AgNPs (AgNPs-K, AgNPs-M, and AgNPs-E) synthesized by bacteria is shown in **Figure 4**, which was used for the identification of various functional groups present in AgNPs. All the samples have a common band at 599 $cm^{-1}$ and 963, 1299, 1349, 1693, 2299, 2891, and 3780 $cm^{-1}$. The band at 599 $cm^{-1}$ is attributed to the metallic Ag. The band at 963 $cm^{-1}$ is attributed to the amide V band arising due to out-of-plane NH bending of peptide linkages (Kalpana & Lee, 2013). A small intensity band at 1051 $cm^{-1}$ is attributed to the primary amine C–N stretch. Another small intensity band at 1349 $cm^{-1}$ in all the samples is attributed to the C-C bond. A small intensity band in all the samples at 1699 $cm^{-1}$ is attributed to the OH group in the samples. Moreover, this band is also attributed to the C=O stretching of amide I bands of peptide linkage. The band at 1229 $cm^{-1}$ is attributed to the CN stretching of peptide linkage. The band at 1349 $cm^{-1}$ is attributed to the (C–C) stretching vibration of aliphatic amines, which was previously documented by Ibrahim and their research group. In their study, the investigators developed AgNPs from endophytic bacteria, which showed a FTIR band at 1359 $cm^{-1}$ (Ibrahim et al., 2019). All the samples exhibit an atmospheric carbon band at 2891 $cm^{-1}$ is attributed to the methylene C–H asymmetric or symmetric stretch. All the samples showing a small band from 3400 $cm^{-1}$ to 3800 $cm^{-1}$ centred at 3780 $cm^{-1}$ are attributed to the -OH molecule.

Earlier, Saleh and Alwan (2020) obtained four distinct peaks for the AgNPs synthesized by *K. pneumoniae* at 3332.78 $cm^{-1}$, 2115.35, 1635.60, and 1096.92 $cm^{-1}$. The investigators concluded that the band obtained at 3332.78 $cm^{-1}$ is due to the stretching vibration of the OH bond of alcohol and phenols. The band at 2115.35 $cm^{-1}$ was found due to the C-H stretching of the methylene groups of protein and to the N-H stretching of amine salt. The band at 1635.60 $cm^{-1}$ was attributed to the carbonyl groups (C=O) of the amino acid residues, while the band at

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1096.92 $cm^{-1}$ was attributed to the (C-O) stretching of alcohols and esters, carboxylic acids, and C–N stretching of aliphatic amines (Saleh & Khoman Alwan, 2020). Based on the above information, it was further concluded that the presence of protein in the supernatant acts as a stabilizing and capping agent for stabilization, which binds to the synthesized AgNPs through free cysteine or amine groups in proteins (Saleh & Khoman Alwan, 2020). Previously, Kalpana and Lee (2013) also obtained bands for the AgNPs synthesized by using a culture of simulated microgravity-grown *K. pneumoniae*. The investigators obtained major intensity bands at 2964.55 $cm^{-1}$, 1262.22 $cm^{-1}$, 1095.89 $cm^{-1}$, 1021.96 $cm^{-1}$, 800.73 $cm^{-1}$, and small intensity bands at 2960.64 $cm^{-1}$, 1650.01 $cm^{-1}$, 865.33 $cm^{-1}$, 701 $cm^{-1}$ and 477.07 $cm^{-1}$.

**FIGURE 4** FTIR spectra of AgNPs synthesized by bacteria.

In the current investigation, authors have also obtained bands for the *K. pneumoniae* synthesized AgNPs at 599, 963, 1229, 1693, 2891, and 3780 $cm^{-1}$ which correspond to the bands obtained by Kalpana and Lee (Kalpana & Lee, 2013). Research group led by Peiris also obtained four prominent FTIR bands at 1643, 1586, 1397, and 1042 $cm^{-1}$ and concluded that the AgNPs synthesized by bacteria have enhanced stability because of the coating of AgNPs by bacterial and media components (Peiris et al., 2018). AgNPs-M and AgNPs-E displayed similar bands as those of AgNPs-K with slight variation in their intensity.

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### 4.4. Phase identification of silver nanoparticles by XRD

The XRD investigation was carried out to identify the crystalline phase of the AgNPs. A typical XRD pattern of all the bacterially synthesized AgNPs is shown in **Figure 5**. All the AgNPs exhibit three characteristic peaks of silver NPs at 27.6°, 32.1°, and 46.2°, and three small intensity peaks at 54.7°, 57.4°, and 76.7°. The major intensity peaks in all three types of bacterially synthesized AgNPs are at 32.1°, followed by 46.2° and 27.6°. The XRD planes in all three types of AgNPs were 101, 111, 200, 220, and 311, as matched with the Joint Committee on Power Diffraction Standards (JCPDS) 03-0921. In the current investigations,

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the authors obtained diffraction peaks at 27.6°, 32.1°, and 46.2° and small diffraction peaks at 54.7°, 57.4°, and 76.7° for the AgNPs-K. AgNPs-M also has the same peaks as AgNPs-K, i.e., at 27.6, 32.1, 46.2, 54.7, and 57.4°. All the XRD peaks for AgNPs-M were of almost the same intensity except for 27.6°, which was comparatively stronger than AgNPs-K. The XRD peaks for AgNPs-E were also at the same places as those of AgNPs-M and AgNPs-K, but the peak at 27.6° was of stronger intensity than AgNPs-K and weaker than the AgNPs-M. Moreover, the peak at 46.2° in both AgNPs-M and AgNPs-E was stronger than the AgNPs-K. Further, the peaks at 54.7° (311) and 57.4° (222) were like a small, broad hump in both AgNPs-K and AgNPs-M, but these two peaks were sharper in AgNPs-E.

The results were in close agreement with the previous results obtained by Kalpana and Lee (2013) and Ibrahim et al. (2019), where Kalpana and Lee (2013) obtained diffraction peaks at 37.76°, 45.87°, 64.08°, and 77.11°, which were indicated by the (111), (200), (220), and (311) reflections of metallic Ag. The data obtained here was matched with the database of JCPDS file no. 03-0921 (Kalpana & Lee, 2013). Ibrahim et al. obtained diffraction peaks for the AgNPs synthesized by an endophytic bacteria, *Bacillus siamensis* strain C1, at 27.81, 32.34, 46.29, 57.47, and 77.69°, corresponding to (101), (111), (200), (220), and (311) crystal planes, respectively, for the AgNPs (Ibrahim et al., 2019). Earlier, a research group led by Vimalanathan also obtained similar results, where the major peaks were at 28, 32, and 47°, and two small intensity peaks at 55° and 57°, and a small diffraction peak at 76°.

**FIGURE 5** XRD pattern of silver nanoparticles synthesized by bacteria.

The crystalline size of all three AgNPs synthesized by bacteria was determined by using the Scherrer formula as given in Equation (2),

$$D = \frac{k\lambda}{\beta \mathrm{Cos}\,\theta} \qquad (2)$$

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Where,

D =crystalline size,

k =constant (0.9), and

β = the FWHM values of the diffracted peaks.

The highest intensity peak was used to find all the parameters in the Scherrer equation. The Gaussian peak fits were used to find the FWHM values and exact theta values. The calculated crystalline size was found to be around 16.88 nm, 18.00 nm, and 16.44 nm for AgNPs-K, AgNPs-M, and AgNPs-E. Therefore, it is well examined that the synthesized AgNPs showed a crystalline nature and 16.88 nm, 18.00 nm, and 16.44 nm crystallite sizes.

**4.5. Morphological analysis of silver nanoparticles by FESEM and elemental analysis by EDS**

**Figure 6a-6f** shows FESEM micrographs of AgNPs synthesized by *K. pneumoniae* (AgNPs-K). **Figures 6a&b** show a porous flakes-like structure that is embedded with bright-colored AgNPs. **Figure 6c&d** clearly shows rhombohedral-shaped AgNPs, whose size varies from 22-66 nm. These two images clearly show that the AgNPs are embedded in porous, sheet-like structures. **Figure 6e&f** show aggregated spherical-shaped structures. Previously, several investigators have shown similar morphology for the bacterially synthesized AgNPs.

**Figure 6g** shows the EDS spot of the AgNPs-K, while **Figure 6h** exhibits the EDS spectra and elemental table of the AgNPs-K. The EDS spectra in **Figure 6h** show peaks for Ag, Cl, Na, P, S, C, O, and N. Among these, the elements contributing the most to the sample were Ag (37.8% At wt.), Cl (29.8%), and P and S were 0.4% each. The P and S were not present in trace amounts in the AgNPs-K. The major impurities in the synthesized AgNPs-K are NaCl, which alone comprises 50%, which is due to the improper washing of the samples. Moreover, these two may also come from the nutrient broth used for growing the bacteria. While the presence of C,

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S, and P indicates the association of biomolecules with the synthesized AgNPs-K. Earlier, Sayyid and Zghair (2021) reported cube-shaped to irregular heterogeneous forms of AgNPs synthesized by *K. pneumoniae*, whose average size was 40.47 nm (Sayyid & Zghair, 2021). Moreover, investigators further observed that the morphology by TEM was a pseudo-spherical shape of size 40-80 nm.

**FIGURE 6** FESEM images (a-f), EDS spot (g), and EDS spectra and elemental table (h) for AgNPs-K. FESEM images (i-l), EDS spot (m) and EDS spectra, and elemental table (n) for AgNPs-M. FESEM images (o-r), EDS spot (s) and EDS spectra, and elemental table (t) for AgNPs-E.

Saleh and Alwan (2020) obtained spherical-shaped particles of size 26.84 to 44.42 nm, which were highly aggregated. Further, the investigators concluded that the conglomeration of the AgNPs occurs during the drying process (Saleh & Khoman Alwan, 2020).

A research group led by Rasheed obtained nano-rod-like AgNPs of size 100-200 nm from the *Conocarpus* erectus plant, while oval-shaped AgNPs of size 110-150 nm were synthesized from *Pseudomonas sps*, and by applying the chemical reduction method, flower-like AgNPs of size 100-200 nm were obtained. So, the smallest size was reported from *Pseudomonas sps*, which was oval-shaped.

**Figure 6i-l** shows FESEM micrographs of AgNPs synthesized by the *M. luteus* (AgNPs-M). **Figure 6i&j** shows a high aggregation of the synthesized AgNPs-M. **Figure 6k** and **Figure 6l** show spherical-shaped AgNPs-M, whose size varies from 21-45 nm. **Figure 6m** shows the EDS spot of the AgNPs-M while **Figure 6n** exhibits the EDS spectra and elemental table of the AgNPs-M. The EDS spectra of AgNPs-M in **Figure 6n** show peaks for Ag, Cl, Na, P, S, C, O, and N. Out of all these, the major elements were mainly Ag (61% At wt.), Cl (21%), C (8.4%), N (3.7%), and O (2.6%). Other detected elements, such as Na, P, and S, were present

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in trace amounts. The major impurity in the final sample was Cl, which is due to improper washing of the sample. Moreover, it also came from the bacterial media i.e., nutrient broth. While the presence of C, N, S, and P indicates the presence of biomolecules with AgNPs-M.

**Figure 6o-r** shows FESEM micrographs of AgNPs synthesized by *E. aerogenes* (AgNPs-E). **Figure 6o&p** shows a porous flakes-like structure that is embedded with the bright color of AgNPs. **Figure 6q&r** clearly shows rhombohedral-shaped AgNPs-E, whose size varies from 24-60. The images clearly show that the AgNPs-E are embedded in the porous flakes-like structures. The particles are showing high aggregation, as evident from the SEM micrographs. **Figure 6s** shows the EDS spot of the AgNPs-E, while **Figure 6t** exhibits the EDS spectra and elemental table of the AgNPs-E. **Figure 6t** shows the spectra of Ag, Cl, Na, P, S, C, O, and N. Out of all these, the major elements were mainly Ag (52.1% At wt.), Cl (21.7%), C (9.8 %), O (8.2%), Na (4.0%), and N (3.1%). In addition to this, P and S were present in trace amounts, whose total composition was near 1.1%. The major impurities in the synthesized AgNPs-E are NaCl, which alone comprises 25.7%, which indicates the improper washing of the sample. Moreover, these two may also come from the nutrient broth used for growing the bacteria. The presence of C, S, N, and P indicates the association of enzymes and proteins from the *E. aerogenes* with the synthesized AgNPs-E. **Table 1** shows the major elements present in all three types of AgNPs synthesized by bacteria.

**TABLE 1 Comparison between all the elements present in all three types of AgNPs.**

From the EDS data of all three types of AgNPs, it was found that Ag was present in the highest percentage in AgNPs-M and at least 37.8% in AgNPs-K. Among all the three types of AgNPs, Cl was present most in AgNPs-K and least in AgNPs-M (21.0%). The oxygen was present in the highest amount in AgNPs-E and the least in AgNPs-K, i.e., 8.2% and 2.8%, respectively. The carbon was highest in AgNPs-E (9.8%) and least 7.0% in AgNPs-K. Out of all the three

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types of AgNPs, Na was highest in AgNPs-K (21.8%) and least in AgNPs-M (0.4%). Among all the three samples of AgNPs, N was present highest in AgNPs-M (3.7%) and 3.1% in AgNPs-E, and it was not detected in AgNPs-K. The P and S were present almost identically in all the samples but least in AgNPs-K, i.e., 0.4%.

In the current investigation, the authors found a broad peak of silver ions at 3 keV in all three types of AgNPs, which confirmed the reduction of $Ag^{+}$ to $Ag^{0}$. Moreover, here the authors have mainly peaks in EDS for Ag, Cl, and carbon. The peaks for Ag, Cl, and S were consistent with the results obtained for the AgNPs synthesized by endophytic bacteria by Ibrahim and his team. Ibrahim and his research group also concluded that the broad peak of silver ions was formed at 3 keV, which indicated the reduction of $Ag^{2+}$ to $Ag^{0}$ (Ibrahim et al., 2019).

**Table 2** shows the major microbial proteins and enzymes present in *K. pneumoniae, M. luteus,* and *E. aerogenes*, while **Table 3** shows the comparative analysis of all the previously reported bacterially synthesized AgNPs with the current investigation.

**TABLE 2 The major microbial proteins and enzymes present in *K. pneumoniae, M. luteus,* and *E. aerogenes*.**

**TABLE 3 The comparative analysis of all the previous studies and current investigations of the bacterially synthesized AgNPs.**

From all the previous investigations, it was revealed that the largest size of AgNPs was 40.47±89 nm synthesized by using *K. pneumoniae*, whose shape was cube to spherical. The smallest AgNPs were synthesized by *B. cereus*, whose size was 2-16 nm and spherical shaped. In the current investigation, the size of the synthesized AgNPs varied from 21 nm to 66 nm. Earlier, three more investigators synthesized AgNPs from different species of *K. pneumoniae*, which were mainly spherical shaped and cube-shaped. Except in one or two cases, most of the synthesized AgNPs were spherical in shape, whereas in two cases, cube shaped AgNPs were

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obtained. In terms of elemental composition and purity, AgNPs synthesized by *B. siamensis* strain C1 were purest, where Ag was 91.8% while the remaining was impurity mainly by Cl and S, whereas in our case the Ag percentage varied from 37.8% to 61.6%, i.e., less than the earlier reported by Ibrahim and his team (Ibrahim et al., 2021). The lower purity could be due to improper washing of the AgNPs during centrifugation. From the UV-Vis study, it was found that the absorbance peak of AgNPs synthesized from different bacteria could be varied from 400 to 510 nm based on the size and shape of the synthesized AgNPs. From XRD and FTIR, it was found that the majority of the peaks and bands remain the same in all the syntheses with slight variations, respectively.

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### 4.6. Batch adsorption study of methyl orange dye by AgNPs

MO dye shows the highest absorbance at 464 nm when examined using a UV-Vis spectrophotometer. The AgNPs-M, AgNPs-K, and AgNPs-E treated MO were measured for their color intensity in an aqueous dye solution for up to 120 minutes at a regular interval of 30 minutes. With passing time, the concentration of MO dye in the sample decreased gradually, which is evident from the UV-visible spectra (Figure 7 a, b, and c). So, the maximum removal of MO dye was found after 120 minutes using AgNPs-M. The readings at an interval of every 30 minutes show a slow decrease in the concentration and absorbance of the dye; hence, the decrease in the graph can be observed easily as it moves from 0 minutes to 120 minutes. **Figure 7 a, b, and c** shows the MO dye absorbance by UV-Vis spectroscopy at different time intervals.

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**FIGURE 7** MO dye removal by different types of AgNPs with respect to contact time as measured by UV-Vis spectrophotometer: a) AgNPs-K, b) AgNPs-M, and c) AgNPs-E.

### 4.7. Percentage removal of MR dye by all the AgNPs

AgNPs-M removed MO dye 2.34% at 30 minutes, 4.37% at 60 minutes, 14.83% at 90 minutes, and 19.24% at 120 minutes. AgNPs-K removed MO dye at 1.5% at 30 minutes, 4.06% at 60

minutes, 9.56% at 90 minutes, and 15.03% at 120 minutes. AgNPs-E removes MO dye 1.52% at 30 minutes, 2.36% at 60 minutes, 3.86% at 90 minutes, and 4.15% at 120 minutes. By comparing all the AgNPs mentioned above, we can observe that AgNP-M has the highest efficiency of dye removal and AgNPs-E has the lowest efficiency of dye removal. **Figure 8** shows the percentage removal of MO dye by all three types of AgNPs.

**FIGURE 8** Percentage removal of MO dye by all three types of AgNPs.

Earlier, Gola and their colleagues successfully utilized AgNPs, synthesized by *Aspergillus sps*., to degrade the reactive yellow dye in an aqueous solution through photocatalysis. Here the initial dye concentration was about 20-100 mg/L, where about 1 g/L of retentate biomass of the fungus removed 82-100% dye, respectively. As the initial dye concentration increased the decolorization efficiency of the biomass retentate decreased from 9.2% to 32.3% (Gola et al., 2021). Rasheed and his research group removed reactive black 5 (RB5), methylene blue (MB), 4-nitrophenol (4-NP), and reactive red 120 dye (RR120) from the aqueous solutions by using AgNPs synthesized from *C. erectus* (plant), *Pseudomonas sps*, and the chemical reduction method. The removal of MB and 4-NP was investigated along with the absence and presence of AgNPs (catalysts) and variable doses of a reducing agent ($NaBH_4$). The removal of RB5 and RR120 dye was carried out in the absence of a reducing agent and variable doses of AgNPs. The bacterially synthesized AgNPs showed almost 100% removal of MB dye within 30 minutes, while AgNPs synthesized by the chemical reduction method showed almost 93.2% removal of RR120, whose more detailed outcomes are compared in **Table 4** (Rasheed et al., 2023). Previously, a research group led by Batool synthesized AgNPs from *Salvinia molesta* and used them for the removal of methylene blue dye from the aqueous solution. Where, the highest adsorption capacity of the dye on the surface of AgNPs was 121.04 mg/g by the Langmuir isotherm (Batool, Daoush & Hussain, 2022). Bhankhar et al. removed MO up to 83% by using chemically synthesized AgNPs in the presence of $NaBH_4$ under optimized

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conditions (Bhankhar et al., 2014). **Table 4** depicts the summarized form of all the investigations and current investigations where AgNPs were used for the removal of MO from wastewater.

**TABLE 4 Summarized form of all the investigations and current investigations where AgNPs were used for the removal of various dyes from wastewater.**

From all the above investigations mentioned in Table 4, it was concluded that previously only one attempt was made to remove MO dye that too by using AgNPs synthesized by chemical reduction method, where the removal percentage was 83% within 2 minutes (Bhankhar et al., 2014). Most of the investigators tried to remove MB dye by using AgNPs synthesized from plants and bacteria by chemical method. There was only one attempt where dyes (MB, 4-NP, RB5, and RR120) were removed by using AgNPs synthesized by *Pseudomonas sps*, where the removal efficiency varied from 40-100% under optimized conditions. The highest MB removal was observed with AgNPs synthesized from *Pseudomonas sps.* in comparison to the AgNPs synthesized from *C. erectus* (100-200 nm) and chemical reduction method (100-200 nm), which could be attributed to the smallest average size (110-150 nm) of the *Pseudomonas sps* mediated synthesized AgNPs (Rasheed et al., 2023). Moreover, in current investigation, AgNPs-M have the smallest average size (21-45 nm), which exhibited the highest MO dye removal, i.e., 19.24% in 2 hours. In our current investigation, the removal percentage of MO was just 4-19.24% which is almost 5 to 15 folds less than the attempt by using AgNPs synthesized by the chemical method. This could be so because, in the current investigation, the dose of the AgNPs was 1 mg/100 mL whereas Rasheed et al used 100 mg/50 mL of AgNPs synthesized from *Pseudomonas sps* for the removal of RB5 and RR120 for the removal of MB, and 4-NP dye (20 mg AgNPs) was used. Zaman and their research team successfully achieved a 94% degradation of MB in an aqueous solution using photocatalysis under solar irradiation (Zaman et al., 2023).

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To achieve a higher removal efficiency of dye, higher dose of AgNPs is suggested. Moreover, further improvement in the dye removal by AgNPs could be done by using different types of chemical reducing agents ($NaBH_4$) along with the AgNPs (Rasheed et al., 2023). However, the utilization of higher doses of AgNPs for the removal of dye at a commercial level from wastewater is economically feasible. From the investigation, it was also concluded that the morphology of the AgNPs also affects the removal efficiency of the dyes, i.e., smaller particles have a high removal efficiency (Zaman et al., 2023), as evident from the current investigation and study done by Rasheed et al. So, to achieve higher efficiency of dye removal, it is advisable to synthesize controlled (small size) and uniform AgNPs. Moreover, the removal of dyes under irradiation (solar, UV, etc.) may increase the removal efficiency using photocatalytic degradation. In addition to this, pH and temperature can be optimized to obtain a higher percentage of dye removal by using bacterially synthesized MO dye in the aqueous solutions.

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### 4.8. Evaluation of the antimicrobial activity of the synthesized AgNPs

AgNPs-K shows a ZOI of 11 mm against *B. megaterium* and *E. fecalis. B. subtilis* shows a moderate ZOI of 10 mm when compared with other zones, and *B. cereus* shows a 9 mm ZOI due to the effect of AgNPs.

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Earlier, Syyaid and Zghair (2021) also used the *K. pneumoniae* synthesized AgNPs for their antimicrobial activity against *S. aureus* and *E. coli*. The investigators used about 40-50 µg/mL of AgNPs and found that *E. coli* was more susceptible to AgNPs compared to Gram-positive bacteria such as *S. aureus*. Further, the investigators suggested that this could be due to variations in the thickness and composition of their cell walls, like peptidoglycan (Sayyid & Zghair, 2021). Saleh and Alwanz (2020) assessed the three concentrations of AgNPs (50, 10, and 150 µg/mL) synthesized from *K. pneumoniae* and evaluated them against *E. coli, P. aeruginosa, S. aureus, and B. cereus*. Investigators reported that the highest concentration, i.e.,

150 µg/mL, was found to be most effective against these pathogens in comparison to 50 and 100 µg/mL. This suggests that the increase in the concentration of AgNPs increases the antibacterial activity (Saleh & Khoman Alwan, 2020).

The use of AgNPs-M for antibacterial activity (Gnanamoorthy et al., 2022) using different bacteria like *B. subtilis, B. cereus, B. megaterium,* and *E. fecalis* shows different ZOI. AgNPs-M shows a maximum ZOI of 12 mm against *B. megaterium* and a minimum ZOI against *B. cereus* of 8 mm. It shows a moderate ZOI of 9 mm against *E. fecalis* and 11 mm against *B. subtilis*. *B. megaterium* was more susceptible than *E. fecalis*, and *B. subtilis* was moderately susceptible, and *B. cereus* was less susceptible to AgNPs-M. *B. cereus* gives a greater ZOI of 11 mm due to the effect of AgNPs-E. The lowest ZOI is observed against *B. megaterium*, which is 8 mm and also shows a 10 mm, ZOI by *B. subtilis and E. fecalis.*

Previously, a research group led by Gola obtained a ZOI of about 13 and 10 mm against *E. coli* and *S. aureus*, respectively, for the AgNPs synthesized by *Aspergillus sps.* Further, the investigator evaluated the synergistic effect of AgNPs and penicillin against *E. coli* and *S. aureus* and showed 0.49- and 0.36-fold increases in the ZOI, respectively, in comparison to the AgNPs and penicillin alone (Gola et al., 2021).

Recently, Haq and Akter (2021) synthesized crystalline, spherical-shaped, 13-27 nm sized AgNPs by using *Paenarthrobacter nicotinovorans* MAHUQ-43. Further, it was evaluated as an antibacterial agent against *B. cereus* ATCC 10876 and *P. aeruginosa* ATCC 10145, where the MIC and MBC for both pathogens were 12.5 µg/mL and 25 µg/mL, respectively. The ZOI at 30 µL against *P. aeruginosa* was 15.5±0.8 mm and 13.6±0.5 mm against *B. subtilis*, whereas at 60 µL the ZOI against *P. aeruginosa* was 24.7±0.9 mm and against *B. subtilis* it was 19.3±1.0 mm (Huq & Akter, 2021).

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A research group led by Ibrahim synthesized spherical-shaped AgNPs of size 5-7.06 nm by using *B. cereus*, whose antimicrobial activity was evaluated against a group of five different bacterial strains, namely: *Staphylococcus epidermidis, S. aureus*, *E. coli, Salmonella enterica, and Porteus mirabilis*, by the agar-disc diffusion method, whose ZOI was 32.12 ± 0.55, 38.25 ± 0.05, 33.05 ± 1.33, 30.73 ± 0.25, and 35.44 ± 1.08 mm at a concentration of about 200 µg/mL of AgNPs (Ibrahim et al., 2021). **Figure 9** shows the antibacterial activity of Petri plates of the AgNPs against tested bacterial species. **Table 5** shows a comparison of the antibacterial activity of the current investigation with previously reported studies.

**FIGURE 9** Antibacterial activity, zone of inhibition of AgNPs synthesized against the tested bacterial species.

**TABLE 5 Summarized form of comparison of antibacterial activity of AgNPs of earlier reported work and current investigation.**

The difference between the antibacterial effect against GPB and GNB bacteria over here might be due to the morphological differences in both types of bacteria (Jubeh, Breijyeh & Karaman, 2020). GPB has a thick peptidoglycan layer, while GNB has a thin peptidoglycan layer but a thick lipopolysaccharide layer (Pasquina-Lemonche et al., 2020). A similar reason was also given by several other investigators for the antimicrobial activity of the synthesized AgNPs (Kalpana & Lee, 2013). Investigators reported that spherical AgNPs have higher antibacterial/antimicrobial activity due to their high SVR (Al-Ogaidi & Rasheed, 2022). Moreover, due to their large surface area, they could bind with various ligands. AgNPs can easily infiltrate the bacterial cell membrane, and that is the reason for their enhanced antimicrobial activity, which might improve more with a further decrease in the size of AgNPs (Anees Ahmad et al., 2020; Wypij et al., 2021). AgNPs get attached to the cell membrane, where they release Ag ions slowly. Further, AgNPs are reported to develop pits in the cell wall

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of microorganisms, which leads to enhanced permeability and leakage of cellular components through the plasma membrane (Tripathi et al., 2017; Matras et al., 2022).

## 5. Conclusions

The shape and method of synthesis of silver nanoparticles can affect their antimicrobial properties. The current study aimed to produce biocompatible and morphologically consistent silver nanoparticles using an environmentally friendly method. Microbial species possess a range of enzymes, such as reductase, that convert metallic silver ions into silver nanoparticles, which is facilitated by bacterial molecules, which act as both capping and reducing agents. In this study, spherical, oval, and porous sheet shapes of silver nanoparticles were successfully synthesized by using different bacterial strains via the green route using a minimal amount of chemicals. The AgNPs synthesised exhibited UV-Vis absorbance maxima at wavelengths of 475 nm, 428 nm, and 503 nm. The XRD analysis revealed the presence of well-defined crystal structures in the synthesised silver nanoparticles (AgNPs), with distinct peaks observed at 26.2°, 32.1°, and 47.2°. Meanwhile, the FTIR analysis detected characteristic bands at 599 $cm^{-1}$, 963 $cm^{-1}$, 1693 $cm^{-1}$, 2299 $cm^{-1}$, 2891 $cm^{-1}$, and 3780 $cm^{-1}$ for all types of AgNPs. AgNPs range in size from 10 nm to several microns, with shapes ranging from spherical to porous sheets, whereas, percentage of Ag varied from 37.8% (wt.%) to 61.6%. The AgNPs synthesised by *Micrococcus luteus* exhibited the highest percentage of methyl orange dye removal, reaching up to 20%, and also exhibited maximum antimicrobial activity against *B. megaterium*, measuring 12 mm. The higher dye removal efficiency and antimicrobial activity of AgNPs-M could be due to the smallest size of the AgNPs produced by *M. luteus*. Therefore, shape significantly influences the ability to remove dyes and antimicrobial properties. Such bacterial-based green synthesis of AgNPs acts as a potential and sustainable approach for material synthesis. Moreover, such approaches could be highly valuable in the field of biomedicine.

**Funding:** This research was funded by the Deanship of Scientific Research at King Khalid University under the grant number R.G.P. 2/174/44.

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**Acknowledgements:** The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for supporting this research work. The authors are also thankful to Department of Life Sciences, Hemchandracharya North Gujarat University for providing the lab facilities.

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