

Biological Synthesis of Silver Nanoparticles by Bacteria and Its Characterizations. A Review

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Abstract:

Background: Silver nanoparticles are the most commercialized nanomaterials, because they are used in a variety of consumer products. Silver nanoparticles can be synthesized by several methods, which are physical, chemical, and biological methods. However, the advantages of biological synthesis method are more environmentally friendly and economical. Biological synthesis of nanoparticles can be done using plants, fungi or bacteria as the bioreductor. In this review article discusses manufacture and characteristics of silver nanoparticles that are synthesized using bacteria. The method used is biological synthesis using bacteria as the bioreductor. Characterization of nanoparticles is important to determine and control the synthesis and applications of nanoparticle. Characterization was carried out using various techniques such as, UV-VIS Spectroscopy, Transmission and Scanning Electron Microscopy (TEM, SEM), powder X-ray Diffractometry (XRD) and Fourier Transform Infrared Spectroscopy (FTIR).

Materials and Methods: The method of work uses literature study by finding sources or literature in the form of primary data in the form of official books and international journals in the last 10 years (2010-2020).

Results: The result obtained are the characteristics of silver nanoparticles in the form of wavelength, shape, size, functional group and crystal structure.

Conclusion: It is known that bacteria act as bioreductors in the synthesis of silver nanoparticles because the secondary metabolites produced by bacteria are responsible for reducing metal compounds into nanoparticles. This can be seen from the change in the color of the silver nitrate solution after being added to the bacterial supernatant culture. The color of the solution changed to brown. Then the results were confirmed using UV-Vis spectrophotometry. If the spectrum results are in the wavelength range of 405-500 nm, it indicates that silver nanoparticles are formed. Then the silver nanoparticles were characterized using SEM / TEM, FTIR, and XRD. Obtained the characteristics of silver nanoparticles are spherical and have a size below 100 nm which were observed using SEM / TEM. The compounds obtained from the FTIR spectrum confirm that the biosynthesis of silver nanoparticles is produced by proteins contained in bacterial cultures which act as capping agents during the production process and prevent the reduction of silver particle agglomeration. The XRD analysis showed that the particles synthesized were cubic structures centered on the surface of the silver nanoparticles.

Key Words: synthesis, characterization, silver nanoparticles, bacteria.

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I. Introduction

Nanoparticles are particles that have a one-dimensional size that is less than 100 nanometers¹. Nanosilver is probably one of the most commercialized nanomaterials. It is used in more than 200 consumer products². Synthesis of nanoparticles by different methods results in varying sizes, shapes, morphology, and even stability. Generally, these methods can be classified into three broad categories which are physical, chemical, and biological (or green) synthesis³.

Basically, there are two approaches for nanoparticle synthesis namely the Bottom up approach and the Top down approach. In the Top down approach, the silver nanosilver reducers involve the mechanical grinding of a bulk piece of the material. The Bottom up approach involves chemical and biological methods to make nanostructures and nanoparticles. These processes involve controlled condensation of solute molecules that are formed during a chemical reaction. The restriction of the condensation or growth leads to the formation of particles of the desired size and shape⁴.

In this article, the method used is the biological synthesis by bacteria method due to the increasing need for environmentally friendly synthesis methods using environmentally friendly reducers and capping agents, such as proteins, peptides, carbohydrates, various species of bacteria, fungi, yeast, algae and plants³.

Biosynthesis of nanoparticles is a kind of bottom-up approach, where the main reaction that occurs is the reduction or oxidation⁵. The microbial enzymes or the phytochemicals with antioxidant or reducing properties are usually responsible for reduction of metal compounds into their respective⁴.

Characterization of nanoparticles is important to understand and control the synthesis and applications of nanoparticles. Characterization is carried out using a variety of different techniques such as UV-Vis spectroscopy, transmission and scanning electron microscopy (TEM, SEM), powder X-ray diffractometry (XRD) and Fourier transform infrared spectroscopy (FTIR)⁶.

II. Methods

In compiling this review article, the technique used is using literature study by searching for literature sources in the form of primary data in the form of official books and international journals for the last 10 years (2010-2020). In addition, in making articles, a review was carried out by searching for data using online media with the keyword being the characterization of silver nanoparticles using bacteria.

III. Result

There are various kinds of manufacturing methods and characteristics of silver nanoparticles. The method used is biological synthesis using bacteria as the bioreductor. The characteristics of silver nanoparticles were carried out using UV-Vis spectrophotometry to confirm the formation of silver nanoparticles which is characterized by a color change in the silver nitrate solution. Nanoparticle characterization included particle size, particle size distribution and functional groups.

The primary references searches that were used in this review article through trustworthy websites such as Scencedirect, NCBI, Researchgate, Google Scholar and other published and trustworthy journals.

Synthesis and characterization of silver nanoparticles:

Table 1. UV-Vis Spectrophotometry, SEM and TEM Results of Silver Nanoparticles

Bacteria	UV-Vis spectrophotometry	TEM/SEM	References
<i>Bacillus methylotrophicus</i>	416 nm	Spherical 10-30 nm	7
<i>Pseudomonas aeruginosa</i>	430 nm	Spherical 8-24 nm	8
<i>Streptomyces albidoflavus</i>	410 nm	Spherical 10-30 nm	9
<i>Bacillus sp</i>	430 nm	Spherical 50-120 nm	10
<i>Bacillus safensis</i> LAU 13	409 nm	Spherical 5-30nm	11
<i>Rhodococcus sp.</i>	420 nm	Spherical 10 nm	12
<i>Aneurinibacillus migulanus</i> 141	405 nm	Spherical 20-25 nm	13
<i>Pseudomonas sp.</i> THG-LS 1.4	412 nm	Spherical 10-40 nm	14
<i>Streptomyces rochei</i> MHM 13	410 nm	Spherical 22-85 nm	15

<i>Pseudomonas aeruginosa</i>	420 nm	Spherical 25-45 nm	16
<i>Bacillus cereus</i> strain GCF112	414 nm	Spherical 16.991 nm	17

Table 2. FTIR Spectrum and XRD Results from Silver Nanoparticles

Bacteria	FTIR spectrum	XRD	References
<i>Bacillus methylotrophicus</i>	-	-	7
<i>Pseudomonas aeruginosa</i>	3422, 2925, 2855, 1736, 1655, 1459, 1379, 1124 and 1046 cm ⁻¹	111, 200, 220 and 311	8
<i>Streptomyces albidoflavus</i>	3296, 2965, 2931, 1724, 1647, 1535, 1452, 1380, 1280, 1230, 1183, 1132, 1098, 1058 and 980 cm ⁻¹	-	9
<i>Bacillus sp</i>	-	111, 200, 220 and 311	10
<i>Bacillus safensis</i> LAU 13	3410, 2930, 1664, 1618, 1389 and 600 cm ⁻¹	111, 200, 220 and 311	11
<i>Rhodococcus sp.</i>	-	111, 200, 220 and 311	12
<i>Aneurinibacillus migulanus</i> 141	3339, 1634 and 669 cm ⁻¹	111, 200, 220 and 311	13
<i>Pseudomonas sp.</i> THG-LS 1.4	3277, 3076, 2961, 2352, 1653, 1594, 1537, 1452, 1403, 1394, 1331 and 1171 cm ⁻¹	111, 200, 220, 311 and 222	14
<i>Streptomyces rochei</i> MHM 13	3420, 2932, 2362, 1639, 1430, 1115 and 613 cm ⁻¹	-	15
<i>Pseudomonas aeruginosa</i>	-	111, 200 and 220	16
<i>Bacillus cereus</i> strain GCF112	1637,65 cm ⁻¹ and 3329,47 cm ⁻¹	-	17

V. Discussions

Nanoparticles are small particles 1 - 100 nm. The formation of nanoparticles is known by the occurrence of color changes in the silver nitrate solution that has been added to the bacterial supernatant culture. Then the results were confirmed by UV-Vis spectrophotometry at a wavelength of 200-800 nm. The results of the analysis using UV-Vis spectrophotometry were displayed with a peak of Surface Plasmon Resonance (SPR). Generally, the SPR peak is influenced by the size, shape, morphology, composition, and the dielectric properties of the environment around the system. The silver nanoparticle colloid is hydrosol, which is the dispersed phase of solids in the liquid dispersing medium. Silver nanoparticle colloids have absorption peak at a wavelength of about 405 - 500 nm¹⁸.

The working method used is biological synthesis using bacteria as the reducing agent. The bacterial isolates used were fermented with silver nitrate (AgNO₃) solution for 48 - 72 hours. Sarina *et al.* (2020) carried out biosynthesis silver nanoparticles extracellularly using endophytic bacterial isolates from surian leaves, in which the bacterial culture in NB media were centrifuged at 8000 rpm for 15 minutes to collect the supernatant. 3 mM AgNO₃ solution was prepared with aquadest. A total 15 mL of 3 mM AgNO₃ was mixed with 5 mL supernatant bacterial culture in a 50 mL Erlenmeyer flask, then in the shaker at a speed of 150 rpm at 37°C and in the dark conditions for 72 hours. Silver nanoparticles were obtained with wavelengths ranging from 405-434 nm¹⁹. Hanifa *et al.* (2020) carried out biosynthesis of silver nanoparticles from surian leaf extract (*Toona sinensis*) and obtained silver nanoparticles with a wavelength of 400-440 nm. In surian leaf extract, phytochemical screening was carried out to identify their secondary metabolites. From the phytochemical test, it is known that surian leaves have phenolic compounds, flavonoids, saponins and triterpenes. Phenolic and flavonoid compounds are groups of compounds that act as primary antioxidants and play an important role in the silver ion reducing properties of extracts²⁰. The reduction reaction in silver nanoparticles is identified by the occurrence of a color change in the solution mixture, which is changed to a brown color, and a constant increase occurs in dark brown. The color change indicates the initial confirmation of the formation of silver nanoparticles because it indicates a reduction reaction from Ag⁺ to Ag⁰ in the presence of the nitrate reductase enzyme that

were found in the bacterial supernatant. This was confirmed by Kumar *et al.* (2007) who extracted a-NADPH-dependent nitrate reductase from *F. oxysporum* and used the pure enzyme to synthesize nanoparticles². Furthermore, these results were confirmed using UV-Vis spectrophotometry. If the spectral peak at a wavelength of 405 - 500 nm indicates that silver nanoparticles are formed. Next, characterization was carried out on silver nanoparticles.

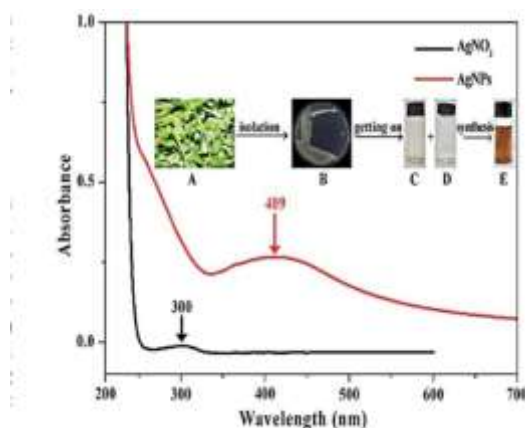


Fig. 1. An Example of Illustrative Image of Synthesizing Silver Nanoparticles. (A): *C. sativum*; (B): Endophytic bacterial isolates; (C): Cell-free supernatant; (D): AgNO_3 ; (E) Reduction of the nanoparticles from Ag^+ to Ag^0 . The synthesis of silver nanoparticles was confirmed by the occurrence of color changes and by UV-Vis spectrophotometric analysis²¹.

In Figure 1 which is an illustrative example of the synthesis of silver nanoparticles using the biosynthetic method using a bioreductor of endophytic bacterial isolates isolated from the *Coriandrum sativum* plant. The synthesis of AgNPs was carried out according to the method by Fouad *et al.*, with a little modification. The bacteria were grown in LB medium at 30°C for 2 days. After that it was centrifuged for 10,000 rpm at 8 minute, 10 mL of culture filtrates were mixed with 90 mL of 3 mM aqueous silver nitrate (AgNO_3) in 250 mL flask and incubated at 30°C for 24 h. The positive synthetic of silver nanoparticles was determined based on the color change to dark brown, then they were analyzed by UV-Vis spectrophotometry and obtained a wavelength of 409 nm²¹.

a. Transmission and Scanning Electron Microscopy (TEM, SEM)

TEM and SEM are surface imaging methods which are fully capable of analyzing different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales. TEM and SEM can determine the morphology of particles and obtain histograms from the images by measuring and counting the particles manually, or by using specific software²².

Based on the shape and the particle size results obtained by each bacteria used shows that bacterial culture can be used to synthesize silver nanoparticles, because they have a size below 100 nm and have a spherical shape. This is in accordance with what was stated by Abdullah *et al.* (2008) which states that the synthesis of nanoparticles means the creation of particles with a size of less than 100 nm and at the same time can change their properties or functions²³.

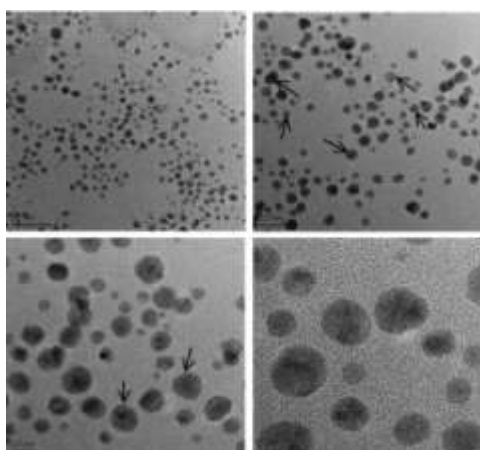


Fig. 2. Example of TEM Images of Silver Nanoparticles²⁴.

b. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) is a tool or instrument used to detect functional groups, identify compounds and analyze mixtures from the analyzed sample without damaging the sample²⁵. FTIR analysis is used to characterize functional groups present in the active precursor compounds and the silver nanoparticles formed. Thus, it can be seen what groups play a role in the reduction and stabilization of silver nanoparticles⁸.

Spectrum result obtained on silver nanoparticles synthesized using culture supernatants *Pseudomonas aeruginosa* strain BS-161R were 3422, 2925, 2855, 1736, 1655, 1459, 1379, 1124 and 1046 cm^{-1} . The absorption bands observed at 1046 cm^{-1} and 1124 cm^{-1} respectively represents the C–O–H bending vibrations and C–O stretching vibrations due to the proteins and rhamnolipids. The absorption bands with wave numbers 1379 and 1459 cm^{-1} corresponds to the bending vibrations of C–H groups of rhamnolipids. The absorption band observed at wave number 1655 cm^{-1} was identified as amide I band which was caused by carbonyl stretch vibrations in the amide linkages of proteins. Furthermore, the signature of stretching vibrations of methylene protein groups which are noticed at the wave number 2925 cm^{-1} . The absorption band at 1736 cm^{-1} is characteristic of the C=O stretching vibration mode of rhamnolipids and the band around 3422 cm^{-1} is almost entirely related to the N–H stretch vibrations of the peptide linkages and the hydroxy stretching vibration of the carboxylic acid groups of rhamnolipid⁸.

Silver nanoparticles that were produced by marine isolate *Streptomyces albidoflavus* obtained spectral results in the vibration band 3296 cm^{-1} representing a primary amine (N–H) stretching, and amide (N–H) bending vibration bands at 1647 and 1535 cm^{-1} . Furthermore, the FTIR spectra of biosynthesized silver nanoparticles were also revealed peaks at 2965 and 2931 cm^{-1} stretching vibrations of aliphatic C–H bonds. A single band presence at 1452 cm^{-1} can be assigned to cut CH_2 stretching vibration in the planar region. Several C–N stretching vibration peaks at 1230, 1183, 1132, 1098, 1058, and 980 cm^{-1} were also observed in the spectral range of 1230 to 900 cm^{-1} . These data further indicate that the isolated marine, *S. albidoflavus* CNP10, produces extracellular protein compound that can bind to synthesized nanoparticles through free amine groups, as well as cysteine residues present in the protein, and thus act as a capping agent during synthesis of silver nanoparticles⁹.

Silver nanoparticles that were synthesized using keratinase from *Bacillus safensis* LAU 13 obtained FTIR spectra 3410, 2930, 1664, 1618, 1389 and 600 cm^{-1} . The peak 2930 cm^{-1} indicated secondary amine, while another peak at 1664 cm^{-1} was recognised as amide I and had been previously shown to be responsible for the capping of silver ion. It has been reported that proteins can bind to silver nanoparticles either through free amine groups or cysteine residues in the proteins. Therefore, it can be concluded that these biomolecules are responsible for capping and efficient stabilisation of the silver nanoparticles¹¹.

Silver nanoparticles that were synthesized using culture supernatant *Aneurinibacillus migulanus* 141, showed that the possible role of biomolecules present in the supernatant responsible for reduction of silver nitrate to silver nanobactericide was assessed with FTIR analysis which showed that the dominant peaks occurred at 3339 cm^{-1} which corresponds to N–H stretching. At 1634 cm^{-1} corresponds to C–N stretching and at 669 cm^{-1} corresponds to C–H stretching¹³.

Silver nanoparticles were synthesized using *Pseudomonas sp.* THG-LS 1.4 shows the FT-IR spectrum recorded from freeze-drying bacterial supernatant powder and silver nanoparticles. The association of amides with amino acid residues in proteins provides a recognized signatures enhancement in the infrared region of the electromagnetic spectrum. The bands seen at 3277, 3076, 2961, 2352, 1331 and 1394 cm^{-1} were assigned to the stretching vibrations of alcohol (O–H), primary amines (N–H), alkane (C–H), amine (C–N), and alcohol (C–O) groups, respectively. Amide bands containing carbonyl groups (C=O) were observed at 1653 and 1594 cm^{-1} . The bands observed at 1452, 1403, 1155 and 1171 cm^{-1} can be assigned to the C–O stretching vibrations of aromatic and aliphatic amines, respectively. These result suggest that a biological molecules might be able to function for the formation and stabilization of AgNPs in an aqueous media¹⁴.

Silver nanoparticles were synthesized using *Streptomyces rochei* MHM 13 to produce FTIR spectra representing intense absorption bands at 3420, 2932, 2362, 1639, 1430, 1115 and 613 cm^{-1} . The intense medium absorbance peak at 3420.14 cm^{-1} (N–H stretch) was characteristic of the amine group¹⁵. Silver nanoparticles synthesized using *Bacillus cereus* strain GCF112 showed strong peaks at wave numbers 1637.65 cm^{-1} and 3329.47 cm^{-1} ¹⁷.

Based on the spectral results obtained by each silver nanoparticles, it showed that there were two types of vibrations (i.e., stretching and bending)⁹. The analysis using FTIR provides insight about the presence of functional groups in the synthesized silver nanoparticles in order to understand how they are able to transform from simple inorganic silver nitrate to elemental silver due to the effect of different photochemicals that might act in a simultaneously as reducing, stabilizing and capping agent¹⁵.

The FTIR spectra stated that the capping agent of biosynthesized nanoparticles have an aromatic amine groups with specific signatures of amide linkages between amino acid residues in the proteins in the infrared

region of the electromagnetic spectrum, as reported by Shaligram *et al.* This type of FTIR spectra supports the presence of a protein type of compound on the surface of biosynthesized nanoparticles, confirming that metabolically produced proteins acted as capping agents during production and prevented the reduced silver particles agglomeration. In fact, carbonyl groups from the amino acid residues as well as peptides are known for strong silver binding property⁹.

Overall observations confirm the presence of protein in silver nanoparticles. It has been previously reported that proteins can bind to nanoparticles via free amine groups or cysteine residues in proteins and, therefore, can provide stabilization of silver nanoparticles. The FTIR studies have confirmed the fact that the amide group form proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium²⁶.

c. X-ray Diffractometry (XRD)

XRD is a primary technique for the identification of the crystalline nature at the atomic scale. The working principle of X-ray diffraction is Bragg's law. Typically, XRD is based on the elastic wide-angle scattering of X-rays²².

XRD patterns were analyzed to determine peak intensity, position and width. Full width at half-maximum (FWHM) data is used with the Scherrer's formula to determine mean particle size. The Scherrer's equation as follows:

$$d = \frac{0.9\lambda}{\beta \cos\theta}$$

where d is the mean diameter of the nanoparticles, λ is the wavelength of X-ray radiation source, β is the angular FWHM of the XRD peak at the diffraction angle θ ²⁷.

Based on the obtained XRD patterns, it shows that each of the spectral peaks at 2θ corresponds to 111, 200, 220 and 311 crystal fields in the biosynthesis of silver nanoparticles. The XRD analysis indicated that the synthesized particles were face centered cubic structure of silver nanoparticles²⁸.

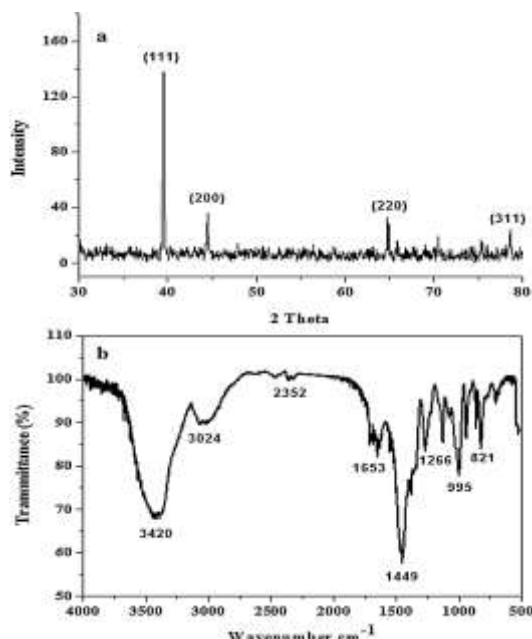


Fig. 3. Examples of X-ray Diffraction Patterns (a) and FT-IR Spectra (b) of Silver Nanoparticles Synthesized With Exopolysaccharide (EPS)²⁴.

V. Conclusion

In conclusion, it is known that bacteria act as bioreducers in the synthesis of silver nanoparticles because the secondary metabolites produced by bacteria are responsible for the reduction of metal compounds into their respective nanoparticles. This can be seen from the change in the color of the silver nitrate solution after being added to the bacterial supernatant culture. The color of the solution changed to brown. Then the results were confirmed using UV-Vis spectrophotometry. If the spectrum results are in the wavelength range of 405-500 nm, it indicates that silver nanoparticles are formed. The analysis results show that the SPR peak is in the range of 405-500 nm. Then the silver nanoparticles were characterized using SEM / TEM, FTIR and XRD. SEM / TEM is useful for seeing the shape and size of silver nanoparticles. Silver nanoparticles are spherical and have a size below 100 nm. Meanwhile, FTIR is useful for seeing what functional groups play a role in the reduction and stabilization of silver nanoparticles. It is known from the results of the FTIR spectrum that aromatic amine groups with specific signs of the relationship between amides and amino acid residues in proteins are involved in the biosynthesis of silver nanoparticles. The compounds obtained from the FTIR spectrum confirm that the biosynthesis of silver nanoparticles is produced by proteins contained in bacterial cultures which act as capping agents during the production process and prevent the reduction of silver particle agglomeration. The XRD analysis showed that the particles synthesized were cubic structures centered on the surface of the silver nanoparticles.

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